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120 Alexander Street, Princeton, N.J.
257 Fourth Avenue, New York 10, N.Y.
25 Hollinger Road, Toronto 16, Canada

FOR THE BRITISH COMMONWEALTH EXCEPT CANADA

CLEAVER-HUME PRESS, LTD.

31, Wright's Lane, Kensington, London, W. 8

VITAMIN A

by

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ELSEVIER PUBLISHING COMPANY

AMSTERDAM . LONDON NEW YORK . PRINCETON

1957

Library of Congress Catalog Card Number 53-7254

With 50 diagrams, 46 plates and 72 tables

PRINTED IN THE NETHERLANDS BY N V DRUKKERIJ G J THIEME, NIJMEGEN

To my Wife, and in memory of my Father,
Professor Benjamin Moore, D Sc , F R S ,
1867–1922

Preface

For nearly 30 years I have been actively engaged in research on vitamin A, and for most of this time have made abstracts of papers by other workers in this field These have been carefully retained and filed away with the idea that they might eventually form the basis for a book For long I cherished the idea that each reliable paper that had made its contribution to progress whether large or small might be adequately mentioned In a work dealing with only one vitamin a fully comprehensive account of all our knowledge *could surely be written without any danger of undue length* I visualised a pleasant spare time occupation which would occupy my leisure for a few months

When my writing had started in earnest however I soon found that the pressure of modern research has made even a single vitamin almost too much for detailed treatment by a single author In the first place the sheer bulk of the available literature would make it difficult to mention every useful paper in the field without turning the text into a monotonous sequence of short abstracts The contents of drawers of reprints several feet thick cannot easily be condensed between the narrow covers of a book The volume of work required occupies not months but years

It is difficult moreover for a single author to follow his subject into several widely different branches of science including chemistry physiology bio chemistry pathology medicine and spectroscopy Critics may consider that each of these subjects should be treated by an appropriate specialist and that it is foolish for one author to attempt work which really requires a team Against this view however it is possible that a book will be better planned and balanced if it is written by a single author than if several specialists make contributions There may be more uniformity in the style of presen tation and better correlation between chapters

In addition to these difficulties which would confront the author of a book on any well known vitamin there are other special problems which are particular to vitamin A These arise mainly from the numerous forms which

the vitamin can assume Since the animal can satisfy its requirements with either preformed vitamin A or with carotene we have, apart from other complications, to discuss the chemistry, physiology and dietetics of at least two distinctly different substances This means that much of our text has to proceed not along a single line, but along parallel lines Thus the problems concerning the assimilation of carotene and of vitamin A from foods are so different as to require discussion in separate chapters Our story of carotene must then be further subdivided for all the other carotenoids capable of acting as provitamins, and our story of vitamin A for its various esters retinene, vitamin A_2 and numerous other derivatives With each of these modifications, moreover, we may have to extend our discussion to cover the effect of *cis-trans* isomerism on vitamin A activity After comparing the activities of α, β and γ carotenes, for example, we must next deal with their respective *neo* isomers

Perhaps some of us may wish that Nature had been less prolific in her network of carotenoid modifications Although they may provide a trial of strength for the organic chemist, and a happy hunting ground for Ph D candidates, they have perhaps tended to distract from the central theme based on the conversion β-carotene to vitamin A_1 We might possibly know more about the mode of action of vitamin A in the body, and about its metabolic products, if all the energy which has been spent on the refinements of carotenoid isomerism could instead have been devoted to these problems But we must accept Nature as we find her If Wald has told us that vitamin A must be converted to a *neo* form before it is incorporated into visual purple we cannot belittle the biological importance of even *cis-trans* isomerism!

Next we must realise that research on vitamin A has not been distributed evenly over the whole possible field, but has tended to be concentrated on certain points which have special scientific, medical or commercial interest. Thus the chemistry and synthesis of the vitamin, its spectroscopic estimation, its role in dark adaptation, its absorption by humans and its importance for cattle have all been the subject of prolific literatures, on which separate monographs might well be written Our chapter arrangements cannot therefore be based on any rigid scheme of dividing and sub-dividing the subject according to chemical, physiological or zoological considerations, but must be guided by the incomplete pattern which research has actually followed At the same time we must, to some extent, condense our account of the crowded and fashionable fields of investigation, and deal more expansively with topics which have been less popular than their importance would have justified

As far as practicable I have tried to trace the historical development of our knowledge of vitamin A in all its aspects To claim infallibility in naming the

originator of every important discovery would certainly invite criticism, particularly from the many authors whose work I have doubtless overlooked I have attempted, however, to give precedence to early work over late work on the same subject as far as the literature has come to my notice The necessity of avoiding loss of knowledge by the relegation of old volumes to the top shelves of the library, or by a habit of confining one's reading to the current numbers of periodicals, has recently been strikingly demonstrated The interesting property of vitamin A in giving a bright blue colour when adsorbed on acid clay has been announced as a new discovery for the third time over a period of 30 years

Finally we may wonder, in following vitamin A through all its interrelationships with physiological and biochemical systems, how far in each circumstance its role can be considered active, as opposed to passive Thus with the vitamin as our viewpoint we may look out upon the activities of digestion, on the intricacies of fat and sugar metabolism, on the interplay of hormones and on all the other bewildering complexities of metabolism When such activities can be correlated with alternations in vitamin A levels, as is often the case, how far can we distinguish between cause and effect? Is the vitamin, in common with many other substances, being swept passively along in the tide of metabolism? Or is it playing some part in controlling the force and direction of the tide? The answer to these questions will obviously depend on the nature of the mechanism concerned Thus in dark adaptation the role of the vitamin is certainly active in the reactions to fever it may well be passive For the present, however, we must often be content to record the changes undergone by the vitamin, in the hope that future research may explain their significance

I must sincerely thank the publishers for their cooperation in producing a long and highly specialised treatise on a single vitamin Perhaps they have been given courage by the success of the excellent monograph on "The Carotenoids" by Professor Paul Karrer and Dr Ernst Jucker, whose lead on an allied subject I am proud to follow With knowledge continually expanding there seems adequate scope for many similar specialised treatises on substances of biological importance

Cambridge, Spring 1957 THOMAS MOORE

ACKNOWLEDGEMENTS AND THANKS

I am grateful to my employers the Medical Research Council for permission to write this book and to my Director Dr LESLIE J HARRIS for his interest and encouragement Most of the manuscript was typed by Mrs MARY HOLMES to whom my special thanks are due After her retirement Misses A C MARTIN J E ETCHELLS and R VARLEY ably completed the work Miss P J HOLDER deserves special commendation for her skill and neatness in drawing numerous figures and graphs Dr V H BOOTH contributed to the Appendix and Mr R J WARD produced the spectroscopic absorption curves required for Figs 3 and 4 Dr I M SHARMAN undertook the heavy task of reading the first proofs As past members of my department Mr A W DAVIES Mrs AILEEN BRIGHT Dr SONIA WALKER and Dr EVA EDEN must be thanked for their part in experiments sometimes unpublished which have been mentioned in this book

Expert advice has been derived from several sources I have been instructed by Professor PAUL KARRER on the numbering of the atoms of the carotenoid molecule and of its double bonds My friend Professor R A MORTON F R S has found me a slow pupil but I trust persevering in understanding the principles of his well known correction procedure in the spectroscopic estimation of vitamin A Professor A W JOHNSON while a fellow member of Christ s College Cambridge helped me with a careful criticism of my account of the synthesis of vitamin A I am indebted to my colleague Dr Z A LEITNER for criticising Chapter 31 and for agreeing to the inclusion of Figs 24 40 and 41 which are based on unpublished data collected jointly with him and with Dr I M SHARMAN For advice as to sources of illustrations I am indebted to Professor H DAM Dr K E MASON and Dr O BLEGVAD Useful comments on my notes on red palm oil (Appendix) were received from Sir LANDSBOROUGH THOMSON C B

Plates 1 2 and 5 were supplied by Distillation Products Industries through the good offices of Drs P L HARRIS and S R AMES Plates 3 and 4 were kindly provided by Hoffmann La Roche & Co Ltd For Plate 6 my thanks are due to Dr HANS POPPER and to the Editor of *Archives of Pathology* Plate 7

has been made up from photographs chosen from a paper by the late Dr S V GUDJONSSON in *Acta Ophthalmologica* The histological sections for Plate 8 were a present from Dr D L WILHELM with microphotography by my colleague Mr K R SYMONDS Illustrations selected from the important papers by the late Sir EDWARD MELLANBY which were published in the *Journal of Pathology and Bacteriology* (Plate 10) and in the *Proceedings of the Royal Society* (Plates 13 and 14) were kindly made available by Lady MAY MELLANBY Plates 11 12 40 41 42 and 43 were contributed by Dr LANE A MOORE and his associates including Dr C F HUFFMAN most of these excellent illustrations have appeared in the *Journal of Nutrition* My colleague Dr J W MILLEN helped with Plate 16

I am much indebted to Dr J WARKANY and to the Editors of various journals for Plate 17 *(Proc Soc exp Biol N Y)* Plate 19 *(J Nutr)* Plate 20 *(Arch Ophthal N Y)* Plate 21 *(J cell comp Physiol)* and Plate 22 *(Pediatrics)* Detailed references will be found in the text Plate 23 appeared in the *Biochemical Journal* Dr F HALE kindly consented to the inclusion of Plate 18 which appeared first in the *American Journal of Ophthalmology* For Plate 24 I am grateful to Dr S Q COHLAN Plates 25 *(J Dairy Sci)* 26 27 28 29 and 30 *(J Hyg Camb)* have been selected from the classical papers of the late Dr C E BLOCH Plate 31 is taken with the permission of Dr LUCIUS NICHOLLS from a communication in the *Lancet* Plate 32 first appeared in the report of the Sheffield Experiment on vitamin A deficiency which was edited by Miss E M HUME and Professor H A KREBS

I am indebted to Indian sources for Plate 33 (Dr M V RADHAKRISHNA RAO and *Indian J med Res*) and Plate 35 (Professor K D LAHIRI and *J Indian med Ass*) Plate 34 appeared in the *British Journal of Dermatology and Syphilis* Dr A E SOBEL and his associates kindly assisted with Plates 36 *(Pediatrics)* 37 38 *(Amer J Med)* and 39 Plate 44 *(Cornell Vet)* was kindly provided by Dr P OLAFSON Plate 45 by Dr S K KON and Plate 46 by Dr W HOHLWEG Quotations of data in tabular form or of the inclusion of modified diagrams have been so frequent that it is impracticable to thank the original authors individually Acknowledgements of the original source of such material however will be found in the text

Finally my thanks are due above all to my wife JANE and family for their constant reminders to get the book finished and for their tolerance of the claims which its writing has made on my leisure hours over several years A happy home life has been the best remedy for the periods of frustration and mental exhaustion which probably afflict most authors who attempt to write learned treatises

T M

Contents

INDEXES

Units and Nomenclature

Units During the long history of research on vitamin A different conventions have been followed in the presentation of quantitative data. Some obsolete units which have survived obscurity by the mist of time, will be mentioned in the main text or in the Appendix. At this point, however, it may be useful to deal with alternative methods of expressing results which still remain in current use.

As will be mentioned in the main text the specimen of carotene which was first chosen as the international standard for vitamin A was later found to be grossly impure. The unit, which had originally been taken as 1 μg of the impure pigment, had therefore to be reduced to 0·6 μg when this first specimen was superseded by pure β carotene. Workers who estimated carotene otherwise than by biological tests were thus given the alternatives of expressing their results in i.u. (0·6 μg units) or by weight (μg or mg units). There was also diversity in the weight of material quoted. Thus scientists might give their results in μg per g, dietitians in i.u. per 100 g and agriculturists in mg per kg (= parts per million) or in mg per lb.

When later the main international standard was changed to preformed vitamin A the unit was taken as an amount of vitamin A acetate which provides 0·3 μg of the vitamin itself. This amount was found to be virtually equivalent to 0·6 μg of carotene, at least according to tests on rats. As with the carotene standard the results could again be expressed either in i.u. or by weight of vitamin A. In practice various methods have been used by different workers, for both carotene and vitamin A, without any formally agreed convention. It is evident, however, that certain combinations of vitamin units and weights of the particular material under investigation have become popular. Perhaps their use may be explained by their obviating the necessity of including unduly large numbers of zeros, before or after the significant digits.

The reader will doubtless be well capable of converting data which have

been expressed in one system into their equivalents in another system The frequent necessity of laborious calculations, however, would certainly not make for easy or pleasurable reading The author has therefore chosen those systems which appear, as far as he can judge, to be the most widely used Results expressed in other systems, by their original authors, have accordingly been recalculated before inclusion in the text The main conventions chosen may be summarised as follows

Form of vitamin A	Material examined	System usually employed
Carotene	Human foods	i u per 100 g
	Animal foods	i u per g or kg
	Blood plasma or serum (experimental)	μg of total carotenoids per 100 ml
Vitamin A	Liver oils	i u per g
	Most other human foods	i u per 100 g
	Animal foods	i u per g or kg
	Liver (experimental)	i u per g
	Blood plasma or serum (experimental)	i u per 100 ml

It will be seen that international units are used except for carotene in blood, which has been a popular subject for research The reason for this decision is that "carotene", as frequently estimated in routine tests, includes other yellow pigments, including xanthophyll, which are inactive as provitamins In the human, when the total carotenoids are low, carotene may virtually disappear from the plasma It might be misleading, therefore, to express the results for total carotenoids in international units, which would imply that all the pigment can serve as provitamin In estimations on foods, on the other hand, the inclusion of inactive pigments in the measurement of provitamins is generally avoided

Nomenclature In regard to nomenclature no particular system has been followed The reader should seldom find difficulty in arriving at the author's meaning, however, if the context is used as a guide Thus "a diet deficient in vitamin A" may be taken, unless otherwise stated, to be deficient in provitamins as well "A diet deficient in preformed vitamin A" will imply that both vitamins A_1 and A_2 are absent, but that provitamins may be present In discussing chemical work, or the visual cycle, vitamin A may usually be identified with vitamin A_1, unless the less familiar vitamin A_2

is specially mentioned Similarly "carotene" will usually imply either a mixture of carotenes or β-carotene, unless the other isomers are named

Karrer's name of "axerophthol" for vitamin A has been used by many workers, and has the advantage of a clear differentiation from carotene It suggests, however, that the action of the vitamin is restricted to the eye In English spelling, moreover, the word "axerophthol" seems unnecessarily cumbrous, and perhaps becomes worse when it is necessary to discuss "axerophthyl" esters Possibly "axerosol" or even "axerol", referring to the general action of the vitamin in combatting xerosis, would have been a simple alternative

In a recent international discussion "retinol" has been proposed as a name for vitamin A It has the advantage of easy spelling and pronunciation, but again it refers to only one function of the vitamin It is perhaps unfortunate that the aldehyde termination "al" produces the same word as the adjective meaning "belonging to the retina". As the title for a paper "The effect of light on the retinal retinal" would be strictly correct, although hardly elegant As another source of confusion Karrer's hydrocarbon 'axerophthene" would presumably fall heir to the name of retinene, which was originally applied to the substance in the retina which eventually proved to be the aldehyde of vitamin A

Numbering of carbon atoms Consistency in the numbering of organic compounds is often difficult, and it is perhaps not surprising that different workers should have adopted widely divergent systems in regard to vitamin A. Danger of confusion, at least to the non-expert, has arisen recently over the numbering of *cis* isomers One school has adopted the same numbering for vitamin A as in the parent carotene from which it is derived (Karrer system) The most common isomerised form of vitamin A, *neo* vitamin A, must therefore be considered as 13 *cis* vitamin A Another school has numbered the vitamin A molecule by the usual convention as an alcohol, starting from the hydroxyl group (Geneva system) According to this scheme *neo*-vitamin A is the 2-*cis* isomer Wald, at one time, numbered the isomers in relation to the double bonds, rather than the carbon atoms In the text the numbering applied by the original authors has been given, but footnotes are inserted if ambiguity is likely to arise

Hemeralopia Confusion may also arise from the wide diversity of terminology used in describing defective vision in partial darkness which is one of the most characteristic effects of vitamin A deficiency The terms hemeralopia, nyctalopia, night blindness and defective dark adaptation are used by different authors as more or less interchangeable descriptions of this condition According to medical dictionaries, however, the correct name for grossly defective dark adaptation is nyctalopia, whereas hemeralopia im-

plies an inability to accommodate the vision to intense illumination. Possibly
the two conditions may to some extent overlap, prior exposure of subject
deficient in vitamin A to intense light certainly aggravates nyctalopia In th
text the terminology of the original authors will usually be followed, and
the reader is warned that the term hemeralopia may not always be used in
a strictly correct sense "Defective dark adaptation" may sometimes imply
no more than its demonstration by experimental methods, without the stag
of clinical night blindness having been reached

PART I
HISTORICAL INTRODUCTION

CHAPTER 1

Early Evidence on the Existence and Nature of Fat-soluble Factor A

Our present information about vitamin A, which is extensive but still far from complete, has been derived through several distinct channels, which have merged their contributions at various points into the general stream of advancing knowledge

Defective dark vision The first recognition of the existence of vitamin A, in the form of a factor capable of correcting an inability to see properly at night dates back several thousand years Thus Aykroyd [1] mentions that Eber s Papyrus an ancient Egyptian medical treatise of about 1500 B C recommends roast ox liver, or the liver of a black cock as curative agents The famous Greek philosopher Hippocrates also prescribed ox liver, but suggested that it should be eaten in a raw state after dipping in honey Modern knowledge of course, has taught us that the livers of almost all animals are rich in vitamin A

As might be expected the importance of a dietary factor in the prevention of certain forms of night blindness has been learnt, by the practical experience of sufferers from the condition in widespread parts of the world still unenlightened by readings from the ancient philosophers Aykroyd tells us that in 1825 Indian Sepoys realised that the affliction was caused in some way by a poor diet Newfoundland fisherman found that their voyages were made hazardous at night by their inability to pick out their course around their rocky coast but were able rapidly to cure themselves by eating livers taken either from cod fish or from sea gulls

Planned scientific research upon the relation of vitamin A to dark adaptation, however, was not carried out until considerable information about its distribution and chemical nature had become available from other sources An important step was then made by Fridericia and Holm [2], who demonstrated that dark adaptation was defective in vitamin A deficient rats, and that the pigment visual purple" could be formed only slowly in their retinas Yudkin [3] proved the presence of vitamin A in the retinas of normal animals and the brilliant investigations of Wald [4] were mainly responsible

References p 8

for our present recognition of visual purple as a protein complex of vitamin A.

Xerophthalmia. The second clue to the existence of vitamin A was also connected with an ocular abnormality, in this case originating on the exterior surface rather than inside the eye McCollum[5] has drawn attention to the work of Magendie, who early in the 19th century found that when animals were restricted to diets composed of such foodstuffs as sugar, starch, olive oil and wheat gluten, now known to be deficient in vitamin A, they developed an abnormality of the eyes which is now known as xerophthalmia Among the early signs of this condition, as usually seen in the rat, is the appearance round the eyelids of a blood-stained exudate, which soon becomes encrusted, and so tends to close the eye Later the cornea is invaded with bacteria, and even if the animal is cured the eye remains permanently damaged

In 1857 the human equivalent of these lesions was noticed in African natives by the explorer Livingstone[6], who remarked that "The eyes became affected as in the case of animals fed pure gluten or starch" Sixty years later the conclusion that the lesions seen in experimental animals were due to deficiency of vitamin A, and not to general malnutrition, was reached by McCollum and Simmonds[7], who adopted the description "xerophthalmia". Extensive studies have subsequently been made on the relationship between xerophthalmia and vitamin A deficiency in human subjects, and in these Bloch[8] played a prominent part.

Fat-soluble factor A The third line of progress, which eventually led to our first hint as to the chemical nature of vitamin A, followed studies of the value of various fats in the nourishment of experimental animals

An early attempt to answer the question whether lipoids in general are essential for normal nutrition was made by Stepp[9] A diet of untreated bread and milk was found to be capable of supporting life in mice for a long period, but if these foods had been extracted with alcohol and ether the animals died within a month Death was prevented by restoring the fatty extract to the animals, but not by supplements of cholesterol, lecithin or kephalin

At about the same time McCollum and his colleagues were engaged in their pioneer researches at the University of Wisconsin, while Osborne and Mendel were progressing along similar lines at Yale Both these groups were primarily interested not in the nutritive value of fats, but in the mysterious dietary ingredients present in natural foodstuffs, later known as vitamins, which were essential as supplements to purified protein, carbohydrates, fats and minerals in order to support life and growth Thus in 1881 Lunin[10] had shown that mice could not thrive on a diet of casein, butter fat and cane sugar even when supplemented with minerals The great British biochemist Hopkins[11]

had mentioned his preliminary findings on the presence in whole milk of "*minimal qualitative factors*" which were necessary supplements to a diet of purified foodstuffs if life and growth were to be maintained

The efforts of the Yale workers were centred on attempts to reproduce the nutritive value of milk by recombinations of its separated components, or of alternative constituents At one point in the complicated advance of their researches they considered that it was unnecessary for fat to be included in the diet but later they concluded that fats were essential Moreover certain fats, such as butter and cod liver oil, had much greater growth-promoting power than others, such as lard and almond oil [12] At Wisconsin the investigations of McCollum were directed mainly towards finding supplements to make good the deficiencies of individual cereal foodstuffs, such as wheat, maize and oats These grains could be made to sustain growth in rats by the addition of supplements of calcium, purified casein and butter fat In comparisons of the effects of various fats as supplements for purified diets egg yolk was found to resemble butter fat in being effective but lard was ineffective [13]

Even butter fat, however, was found to be ineffective in supplementing a diet of polished rice, casein and minerals unless in addition an extract of wheat germ was given In 1915 McCollum and Davis [14] took the initial step in the long journey towards the subdivision of the vitamins by postulating the existence of two factors Fat Soluble A and Water Soluble B

The first chemical clues

The same workers [15] had also been responsible for a valuable hint on the chemical nature of the fat soluble factor when they found that it resisted the action of alkali, and could be recovered in the unsaponifiable fraction after the hydrolysis of active fats Osborne and Mendel [16] supplemented these observations by treating butter for 2½ hours with steam, which had no effect on its growth-promoting powers

Against this impressive evidence of chemical stability, however, Steenbock Boutwell and Kent [17] found that biological activity was lost by heating to 100° C Experiments by Hopkins [18], in one of his few excursions into the vitamin field after his first classical discoveries, not only explained the apparent contradiction, but also revealed another important fact It was true that the vitamin could withstand heat, and in his experience butter fat resisted a temperature of 120° C for 12 hours without loss Rapid destruction occurred, however, when heat was combined with aeration, and no activity was left after air had been passed for 4 hours through the heated fat Zilva [19] demonstrated that oxidation by ozone also rapidly destroyed the vitamin.

The great instability of vitamin A to oxidation was later used by McCollum [20], by this time at Baltimore, to prove its difference from the anti-

References p 8

rachitic vitamin D Cod liver oil which was heated and aerated until it had lost all its anti xerophthalmic and growth promoting power was still effective in curing rickets

Biological activity and yellow pigmentation A very obvious property of butter and egg yolk fats two of the first sources of fat soluble factor A to be investigated was their bright yellow colour On the other hand inactive fats such as lard or almond oil were almost colourless Steenbock [21] concluded that this relationship was more than accidental His belief in this deduction was doubtless strengthened by the findings of McCollum Simmonds and Pitz [22] and of Osborne and Mendel [23] that numerous green vegetables such as cabbage spinach lucerne clover and Timothy grass all possessed high activity as sources of vitamin A It was already well known of course that the predominating bluish green colour of chlorophyll concealed the presence of the yellow lipochrome pigments carotene and xanthophyll (Table 1)

TABLE 1

ASSOCIATION OF VITAMIN A POTENCY WITH YELLOW COLOUR IN FOOD

Active (yellow)	*Inactive or low potency* (white)
Butter	Casein
Egg yolk	Egg white
Cod liver oil	Lard
Yellow maize	White maize
Carrot	Parsnip
Sweet potato	Ordinary potato
Red palm oil	Palm kernel oil
Outer green leaves of cabbage	Inner white leaves of cabbage
Tomato	Onion
Apricot	Apple

Steenbock went so far as to test the biological activities of carotene which he found to be active and of xanthophyll which he found to be inactive He realised however that some fats had high biological activity with little or no yellow pigmentation and to explain this anomaly he assumed that some carotenoid must retain its activity when converted into a hypothetical leuco form

Readers already conversant with the main facts of the interrelationship between the carotenoids and vitamin A will realise ... e conclusions come very near the truth and ... r why the ... ally accepted only after a further 10 yea... e research ... son for this delay in acceptance as far ... after a l ... as was the

uncertain stability of specimens of carotenoids throughout the period necessary for biological tests, which made the results irregular The absence of quantitative data either on biological activity or on the amounts of the substance concerned as estimated chemically also retarded progress

The formation of "vitamin A" in plants

As far as plant sources were concerned, however, the association between vitamin A activity and the carotenoids remained undisputed Steenbock and Boutwell [24] observed that yellow maize was active whereas white maize was not Of a series of roots and underground stems the yellow carrot and sweet potato were active but the common potato, parsnip, mangold, red and sugar beets, rutabaga and dasheen were inactive [25] Coward [26] followed the relationship in a wide variety of plant tissues, including garden flowers and other materials not generally used as foodstuffs. In the "Pheasant's Eye" Narcissus (*Narcissus poeticus*), for example, she found that the orange coloured central corona was highly active, but that the surrounding white petals were inactive Drummond and Coward [27] reported that the highly pigmented red palm oil, unlike most vegetable fats, was a good source of activity

Opinions were more divided as to whether exposure to light was necessary for the formation of the vitamin In a series of investigations on wheat shoots and other seedlings Coward [28] was unable to detect growth-promoting activity in plants which had been grown in the dark, but she found high activity after they had been exposed to light Since chemical tests based on the chromatographic procedure devised by the Russian botanist Tswett [29] indicated that both carotene and xanthophyll were present in the etiolated tissues [30] it appeared that the vitamin could not be identified with either of these pigments The possibility remained, however, that they might be associated with its formation in the plant under the action of light This idea was attractive, of course, both because of the roles which the carotenoids must play in photosynthesis and because sunlight had been found capable of curing rickets Wilson [31], however, was able to demonstrate biological activity in etiolated wheat shoots when a sufficiently high allowance was given Five years of intensive experimentation followed before this conclusion was finally accepted by Coward [32] and confirmed by Moore [33].

Depigmented chickens

In the meantime a demonstration by Palmer and his colleagues of the ability of birds to thrive on a diet from which carotenoids were almost completely excluded had shaken confidence in the theory of a simple relationship between vitamin A and the carotenoids In collaboration with Eckles [34] he had proved some years previously, that the yellow "lipochrome" pigments of animal fats are not produced by the animal, but are absorbed from vegetable sources of the pig-

References p 8

ments present in the diet. This transfer of pigments from the plant to the animal must certainly have suggested strongly that they were physiologically indispensible to animals, a possibility which would not be difficult to put to a practical test.

Palmer and Kempster [35] therefore planned an experiment in which white leghorn chicks were fed upon a diet of white maize, white summer squash and white onions With these foodstuffs the chicks would not grow, but growth and health were maintained by the addition of small supplements of pig's liver, which Palmer had previously found to be virtually devoid of lipochromes Maturity was attained in a normal manner except for a complete bleaching of the skin, beaks, ear lobes and scales of the shanks, which were all bright yellow in birds receiving their usual food Some 800 eggs with colourless yolks were produced by pullets reared on the lipochrome-free diet, and colourless chicks were hatched from them, and in turn raised to maturity on the same diet Before the true significance of the supplement of pig's liver was known it appeared that Steenbock's theory had been put to a fair test, and had been found to be incorrect The existence of a leuco compound derived from the lipochromes could hardly be accepted without some solid proof of its reality

But although the end of this chapter concludes on a note of perplexity considerable knowledge on fat-soluble A had already been gained Thus its deficiency was known to be responsible both for defective dark adaptation and for xerophthalmia It was present in certain fats, but not in others, and also in green and yellow vegetables It survived the treatment of fats with alkalies, but was unstable to oxidation. A good start towards its identification had obviously been made

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CHAPTER 2

The Recognition of Preformed Vitamin A as Distinct from its Carotenoid Provitamins

The H_2SO_4 colour test for liver oils

After Palmer and Kempster had given such conclusive proof that chickens were able to thrive upon diets free from carotenoids there was little occasion for surprise when Drummond [1] announced that he was unable to detect any biological activity in a specimen of carotene isolated from carrots Stephenson [2], moreover, not only confirmed this finding but demonstrated that butter fat retained its biological activity after decolourisation with charcoal. In collaboration with Rosenheim [3], however, Drummond shortly afterwards directed attention to the possible presence of an unknown class of lipochromes in liver, and in so doing made one of the most crucial steps in the investigation of vitamin A

The carotenoids had long been known to give colour reactions, generally blue when treated with concentrated sulphuric acid Fat technologists had also long been aware that cod liver oil could be distinguished from many other oils by its ability to give a bright purple colour when treated with the same reagent Although this reaction was regarded by technologists only as an objectionable feature, indicating a poor technique in extracting the oil [4] (see also Chap 7), Rosenheim and Drummond correctly inferred that the purple colour was a direct indication of the presence of the vitamin

Strong support for this view was soon advanced by Drummond and Watson [5] The reaction was given by the liver oils of fish, birds and mammals, and also more faintly by butter fat and the body fats of some animals In striking agreement with the behaviour of vitamin A the property to produce a colour was lost when the oils were heated and aerated, but not when they were heated in the absence of air On the hydrolysis of the fat the chromogen accompanied vitamin A into the unsaponifiable fraction The livers and fat of pigs and rats which had been made deficient in vitamin A did not give the reaction, but the chromogen was present in animals which had been made deficient and then cured

The high potency of cod liver oil

Up to this point biological tests had been little more than qualitative, and butter fat had not been deposed from the position in which the early developments of research had placed it as the foremost source of vitamin A Even before vitamin A had been differentiated from vitamin D, however, Zilva and Miura [6] devised a method of assay, depending upon the restoration of growth in young rats, which indicated clearly that a daily dose of 2 mg of cod liver oil was equivalent to about 200 mg of butter

This important advance opened up the interesting question of the source from which the cod could acquire so much vitamin A, and led on to studies of its transfer from one species to another Jameson Drummond and Coward [7] found that the marine diatom, *Nitzschia closterium*, was a rich source According to Drummond, Zilva and Coward [8] small shrimps and other plankton consumed the diatoms before themselves becoming the food of small fish, such as the caplin, which were in turn preyed upon by the cod or other large fish

It must have been clear, even to the naked eye, that specimens of cod-liver oil which were 100 times higher than butter fat in biological potency were not 100 times more intensely yellow coloured The evidence that cod-liver oil was much more potent than previously had been realised therefore seemed further evidence against the identity of the growth factor with one of the known carotenoid pigments If Rosenheim and Drummond had been correct in ascribing the colour reaction of liver oils to an unknown carotenoid pigment it was obviously much less intensely yellow coloured than the carotenoids already known

Attempts to isolate vitamin A

The discovery of high activity of cod liver oil prompted two groups of workers to attempt the isolation of the vitamin In Japan as early as 1922 Takahashi [9] prepared concentrates from this material, and later gave a detailed description of 'biosterin' which he claimed to represent the vitamin in pure form [10] He correctly concluded that the vitamin contained only the elements carbon, hydrogen and oxygen, and that it was an unsaturated alcohol which was capable of forming artificial esters and of resisting distillation *in vacuo* without destruction He studied the colour reactions with sulphuric acid and with fullers' earth, and compared them with those given by carotene, cholesterol, 'oxycholesterol'' and dehydrated cholesterol He assumed that biosterin was a carotenoid, but pointed out that unlike most carotenoids it had no selective absorption spectrum in the visible region In the ultra-violet region, however, he detected a band at 320 mμ, which is reasonably close to the maximum at 328 mμ now accepted for pure vitamin A Biosterin also exhibited a beautiful fluorescence when exposed to ultra-violet irra-

References p 16

diation, and was toxic when given to rats and mice in greatly excessive doses. Subsequent research has confirmed that pure vitamin A has all these properties Takahashi's formula of $C_{27}H_{24}O_2$, however, was far removed from that of the pure vitamin, as also was the formula $C_{27}H_{46}O_2$ which was advanced for a less unsaturated form of biosterin which he claimed to have isolated from clover.

While the Japanese work was proceeding Drummond, Channon and Coward [11] had been labouring for five years at University College, London, without achieving, according to their own modest estimate "any success worthy of note" After the hydrolysis of cod-liver oil they removed cholesterol from the unsaponifiable fraction by crystallisation, and also isolated oleyl and selachyl alcohols, the unsaturated hydrocarbon squalene, and unknown saturated and unsaturated alcohols, all of which were found to be biologically inactive Eventually by vacuum distillation they obtained pale yellow oils, which they rightly regarded as highly impure concentrates of the vitamin

On the basis of their careful studies Drummond and his colleagues challenged Takahashi's claim to have isolated pure vitamin A The criticism was certainly amply justified It does appear, however, that the Japanese workers were fortunate in using as their starting material oils which were considerably richer than those available to Drummond, and so were able to finish with more highly potent concentrates Thus the final British products supported good growth in rats in daily doses of 50 μg, in comparison with doses of only 1–5 μg claimed for the Japanese concentrate Drummond, moreover, was unable to repeat Takahashi's observation that large doses of vitamin A were toxic, which suggests that his preparations may have been insufficiently potent to have caused injury. Later developments now soon to follow, on the distinction between vitamin A and its carotenoid precursors, however, have clearly indicated that Takahashi's knowledge of the chemical nature of his concentrates could not have been very profound. If his concentrates had even approached a state of purity he would certainly have found much more disparity than a difference in iodine value between his preparations made from cod-liver oil and green leaves

The arsenic and antimony trichloride reactions

The sulphuric acid colour reaction for vitamin A had the disadvantage that the colour produced was very transient, and was difficult to produce regularly at its full strength. An important advance was made, therefore, when Rosenheim and Drummond [12] found that by substituting arsenic trichloride, a reagent previously used in sterol tests, a much more stable bright blue colour was readily and consistently produced Even with this improvement, however, the colour faded too rapidly for matching in the

types of colorimeter which were then available Rosenheim and Schuster [13] therefore resorted to the Lovibond Tintometer, an instrument consisting of sets of graded blue, yellow and red glasses, which had long proved its value in industrial applications A specially modified instrument, in which the glasses were mounted in frames for rapid handling, allowed the colour reactions of specimens of cod liver oil to be compared with reasonable accuracy

In spite of the brilliance of the colour which it produced, however, arsenic trichloride had certain disadvantages including some danger to the health of the user After an extensive search for a reagent free from these objections Carr and Price [14] found that a saturated solution of antimony trichloride in chloroform gave with cod liver oils a blue colour similar in shade and intensity to that given by arsenic trichloride Carr and Price also adopted the Lovibond Tintometer for matching the intensities of the colours produced by different oils

Further biological tests with carotenoids

In connection with their work on the concentration of vitamin A Drummond and his colleagues retested the biological activity of carotene A specimen which had repeatedly been recrystallised was again found to be inactive, but the survival periods of deficient rats were sometimes increased when less pure specimens of the pigment were administered It appeared, therefore, that the vitamin was present as an impurity Tests on lycopene, the pigment of the tomato were negative Xanthophyll, tested by Willimott and Moore [15], was also ineffective

In 1928, however, the whole question was reopened by a confident report from v Euler, v Euler and Hellstrom [16] that carotene was active in curing rats deficient in vitamin A when administered in doses of about 5 μg daily The Swedish workers pointed out, as was already known, that the carotenoid pigments resembled vitamin A in giving blue colorations with the antimony trichloride reagent They suggested that the failure of previous biological tests with carotene had been due to the omission of vitamin D from the basal diet

Moore [17] confirmed that crystalline carotene had biological activity, but emphasised that it was quite a different substance from the vitamin A of cod liver oil A study of carrot roots indicated that they were a much richer source of vitamin A activity than had previously been supposed, and that the biological activity was concentrated with the carotene during the isolation of the pigment [18] Carotene isolated from red palm oil was active in the same doses as were tried for carrot carotene [19] Further independent evidence of the activity of the crystalline pigment was soon supplied by Collison, Hume, Smedley-MacLean and Smith [20] and by Kawakimi and Kimm [21].

References p 16

The purity of carotene specimens

Further tests with highly purified carotene by Dulière, Morton and Drummond [22], however, were again negative, and these workers therefore suggested that specimens of the pigment were only active when they contained the vitamin as an impurity In a careful colorimetric and spectroscopic differentiation of carotene from the vitamin A of cod-liver oil they found, in confirmation of von Euler and his colleagues, that the blue colours produced with antimony trichloride were of different shades Thus the blue given by carotene had an absorption band at 590 mμ, as compared with 608–612 mμ for the blue given by vitamin A In the ultraviolet region vitamin A was associated with a band at 320–330 mμ which was not shown by carotene It seemed certain, therefore, that carotene and the vitamin were different substances Attempts to find spectroscopic evidence of traces of vitamin A in impure specimens of carotene, however, were unsuccessful It was assumed that the strong absorption of carotene both before or after treatment with antimony trichloride, was so intense as to conceal the presence of the vitamin [23]

As a test of the correctness of this conclusion Moore [24] compared the intensities of the colour reactions given by biologically equivalent doses of carotene and vitamin A, and found that the blue colour of carotene per unit of biological activity was somewhat less than that given by vitamin A The colour produced by carotene, therefore, obviously could not mask the colour reaction of sufficient vitamin A to be responsible for its biological activity It was significant moreover, that when carotene was isolated from the lipoids of the carrot the biological activity had been concentrated in the crystalline pigment and not in the mother liquor which should presumably have been richer in associated impurities

The final settling of the controversy, however, was left to Hume and Smedley MacLean [25] As a precaution against his rats receiving even traces of vitamin A in any form other than the substance to be tested Drummond had decided to dispense with natural vegetable oils as diluents for his test doses, and had made up his solutions of carotene in ethyl oleate Miss Hume and her colleague found that when carotene was dissolved in a natural oil it retained its yellow colour without any obvious loss for a long period Solutions made up in ethyl oleate, however, began to fade in a few days, and soon completely lost their colour In Drummond s tests therefore, the carotene had presumably been destroyed by oxidation before the rats had been dosed for long enough to effect cures

The conversion of carotene to vitamin A

It had become quite obvious at this point, that vitamin A deficiency in rats could be cured equally well by a highly pigmented hydrocarbon, which was readily crystallisable from plant extracts, or by a much less highly coloured

substance, probably an alcohol, which had stubbornly resisted attempts at its crystallisation from liver concentrates. Von Euler and his colleagues, who recognised that the antimony trichloride reaction given by the factor in fish-liver oils was more intense than that given by carotene, suggested that the oils might contain an unknown carotenoid derived from brown or red seaweeds.

In the light of modern knowledge that all the fat-soluble vitamins exist in alternative forms this theory might have seemed attractive At the time, however, it was difficult to believe that two substances, which differed so widely in their physical properties, could have the same physiological activity unless they were closely related chemically Moore [26] therefore devised experiments in which rats were first given a diet deficient in vitamin A, so as to exhaust their livers of the substance responsible for the antimony trichloride reaction, and were then cured by large doses of carotene. Balance sheets were kept of the natural yellow and blue antimony trichloride units which were ingested in the form of carotene, and of the yellow and blue units which were found in the livers after the animals had been killed. The position of the absorption maxima given by the carotene and liver oil were observed in the antimony trichloride reaction, and Capper [27] made corresponding studies in the ultraviolet region The results, which demonstrated clearly that carotene was converted to vitamin A, were summarised as follows:

Carotene	*Vitamin A*
Synthesised in plant	Stored in animal
Intensely yellow	Almost colourless
328 mμ absorption band absent	328 mμ absorption band present
Greenish blue $SbCl_3$ reaction at 590 mμ	Vivid blue $SbCl_3$ reaction at 610–630 mμ

These observations have generally stood the test of time In the light of further experience, however, the description of the colour given by carotene with antimony trichloride as a "greenish blue" requires modification. The reaction of carotene with the reagent is much slower than that of vitamin A, and in the early stages the colour appears greenish through the persistence of unchanged carotene When fully developed the colour is duller, but not greener, than that given by vitamin A.

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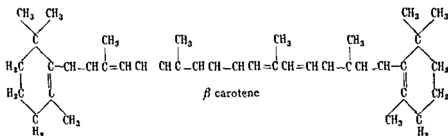
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CHAPTER 3

Chemical Studies of Vitamin A and the Carotenoids

The chemical structures of carotene and vitamin A

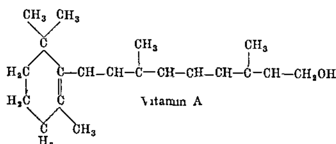
Before von Euler's investigations had intensified interest in the physiological importance of carotene Paul Karrer, of Zurich, had already commenced his classical work on the distribution and chemical nature of the carotenoid pigments By a detailed study of the degree of unsaturation and oxidation products of β carotene, the most important of the several precursors of vitamin A which are now known, he soon succeeded in establishing its structural formula It will be seen that it is a highly unsaturated hydrocarbon $C_{40}H_{56}$ consisting of two terminal aromatic ring structures connected with a long central unsaturated chain [1, 2]



In commencing a similar investigation of vitamin A Karrer and his colleagues enjoyed a great advantage over previous workers who had tried to isolate the vitamin, since the Schmidt Nielsens [3] had recently found that its concentration in halibut-liver oil was 25 to 50 times higher than in cod-liver oil Thus the crude starting material had a potency about equal to the unsaponifiable fractions which Drummond and Takahashi had so laboriously prepared Preliminary trials in collaboration with von Euler [4] fully confirmed the value of the new source, and Karrer, Morf and Schopp [5] soon succeeded in preparing much richer concentrates than had previously been available By saponifying the oil, freezing out inactive substances from the unsaponifiable fraction, and submitting the product to chromatographic absorption on fullers' earth, concentrates active in rats at daily doses of 0 5 μg were

obtained Although the vitamin still resisted crystallisation it is now clear that substantially pure preparations were obtained

Chemical methods similar to those used for carotene eventually led Karrer to a formula of $C_{20}H_{30}O$ for vitamin A The structure it will be seen repre



Vitamin A

sents one half carotene molecule combined with one molecule of water so as to form a terminal hydroxyl group Estimations of molecular weight however had at first suggested a C_{22} formula with a correspondingly longer side chain and this alternative possibility now known to be incorrect could not for a time be ruled out

The carotene isomers and other provitamins

While Karrer s studies on the chemical nature of carotene and vitamin A were in progress evidence became available from several sources to show that carotene exists in different isomeric forms [6 7 8] Thus when carotene ob tained from carrots was dissolved in carbon disulphide and iodine was added optically inactive β carotene was precipitated leaving the strongly dextro rotatory α carotene still in solution In carotene as isolated from different sources the proportion of the α form varied being 10–20% in specimens made from carrots up to 50% in specimens from red palm oil and nil in spe cimens made from grass or stinging nettles

The discovery in carotene of a small fraction distinguished by the physical characteristic of optical activity must have reminded many workers of the spectroscopic detection of 7 dehydrocholesterol which acts as a provitamin D as an impurity in cholesterol In this instance however history was not repeated Kuhn and Brockmann [9] found that both the β and α forms were biologically active In both promoting growth and causing the storage of vitamin A in the liver however the β form was considerably more active than the α form This difference may be simply explained by β carotene having the ring structure necessary for the formation of vitamin A at both ends of its molecule whereas α carotene only has the required formation at one end

Several other carotenoids capable of acting as precursors of vitamin A will be mentioned later (Chap 8) In the meantime it may be emphasised that repeated tests have shown that the ability to act as provitamins is not a

general property of the carotenoid pigments In particular the common pigments xanthophyll and lycopene are quite inactive

The estimation of vitamin A Even before the existence of the various isomeric forms of carotene had been recognised preliminary steps had been taken to set up a standard substance for use as a yardstick for vitamin A estimations Since at the time the reliabilities of both the antimony trichloride and spectroscopic methods were still open to question the proposed standard had only to be considered for its suitability for use in biological tests

Carotene had an advantage over preformed vitamin A in being readily obtainable in crystalline form and in 1931 the Permanent Commission on Biological Standardisation of the League of Nations adopted as its standard a stock of carotene which had been collected from various laboratories [10] Soon afterwards it was realised that this standard was impure and with the substitution in 1934 of pure β carotene as the standard substance the size of the unit was reduced from 1 μg to 0 6 μg to allow continuity [11]

During the past 20 years all the available methods for the estimation of vitamin A have been studied intensively and great pains have been taken to ensure the best possible agreement between the results found by different techniques These will be discussed in detail later but for the present it may be said that the most urgent problem has been to find methods for interrelating the activities of preformed vitamin A particularly estimated by absorption at 328 mμ and its carotenoid precursors To some extent however this step has recently been circumvented by the adoption of vitamin A acetate which is now readily available as the standard for preformed vitamin A with a unit of 0 0344 μg [12] For the estimation of provitamins the carotene standard is still retained

The isolation and synthesis of vitamin A For several years after Karrer had deduced the correct structural formula of vitamin A repeated attempts at its crystallisation were all unsuccessful In 1935 however Hamano [13] succeeded in crystallising the β naphthoate ester of vitamin A which he proved to be physiologically active Two years later Holmes and Corbett [14] went further in crystallising the vitamin itself in the form of pale yellow needles from liver oils obtained from three different species of fish Crystallisation was induced by adding small amounts of water to solutions of the concentrates in methanol followed by freezing and the removal of crystals at a low temperature The crystals so obtained had melting points of only about 7–8° C and subsequently were found to contain solvent By cooling ethyl formate solutions of concentrates obtained by molecular distillation Baxter and Robeson [15] readily obtained yellow solvent free crystals which melted at 63–64° C

References p 21

The synthesis of vitamin A was also delayed until long after knowledge of its structural formula was available Numerous early attempts were made from about 1931 with β ionone, $C_{13}H_{20}O$, a ketone having the same skeleton as the end ring of the vitamin, as the starting material Valuable exploratory work was carried out by Heilbron and his colleagues [16, 17, 18] but the first claim to have achieved the synthesis was made in 1937 by Kuhn and Morris [19] Only a few milligrams of impure material were obtained, which was claimed to be biologically active Other investigators were unable to repeat Kuhn's procedure, and it was not until ten years later that Arens and Van Dorp [20] and Isler, Huber, Ronco and Kofler [21] succeeded in producing the vitamin in substantial quantities in pure form Recently several others workers have described alternative routes of synthesis, and the pure vitamin is now successfully manufactured on a commercial scale

The synthesis of carotene, and its chemical conversion to vitamin A

The synthesis of carotene also took many years to accomplish, but eventually in 1950 Karrer's brilliant and prolonged studies of carotenoid chemistry were crowned by success in the total synthesis not only of β-carotene [22] but also of lycopene [23] and several other pigments

It might certainly have been expected that progress towards the synthesis of vitamin A and carotene would be slow and arduous The conversion of carotene to vitamin A, however, appeared to be such a simple problem that little difficulty could at first have been anticipated in replacing the action of the animal body either by chemical treatment or by an enzyme system In practice, however, the conversion remained unaccomplished for about 15 years Presumably this was due to its taking place, at least *in vitro*, not in a single stage by adding water but by oxidation followed by reduction In 194[illegible] Hunter and Williams [24] made the crucial observation that by treatment with hydrogen peroxide β carotene could be broken in two and oxidised to vitamin A aldehyde, but the yield was only about 0 5 % Various other workers made improvements by the use of catalysts, but eventually Meunier, Jouanneteau and Zwingelstein [25], reported a much more effective method Ball, Goodwin and Morton [26] had already found that when vitamin A was adsorbed upon manganese dioxide it was readily converted to vitamin A aldehyde Meunier and his colleagues now claimed that the same product was obtained from carotene, in yields of 60–80 % The identity of the vitamin A aldehyde was confirmed by reducing it to the vitamin with lithium-aluminium hydride These claims, however, lack confirmation

Vitamin A_2

An interesting diversion of interest from the main line of research on vitamin A was opened up in 1937 by the discovery of a new form of the vitamin characteristic of the livers and other tissues of

fresh water fish The existence of this modification was inferred by Lederer and Rosanova [27] from the abnormal colour reaction given in the antimony trichloride reaction by the liver oils of fish caught in the Murmansk sea Study of the properties of the new factor was made difficult by the simultaneous presence of the ordinary form of the vitamin from which it could not readily be separated At the Liverpool school Edisbury Morton and Simpkins [28] made similar observations finding the highest proportion of the new form in the eyes of gold fish

Vitamin A_2 was eventually isolated in the form of its crystalline aldehyde by Salah and Morton [29] and synthesised by Farrar Hamlet Henbest and Jones [30] It differs from vitamin A_1 only in having one more double bond Its mode of formation in fish remains uncertain but apparently it cannot be formed from dietary supplies of vitamin A_1 It promotes growth in deficient rats and produces a modified form of visual purple in the dark adapted retina

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CHAPTER 4

Lines of Development

It has been possible, up to this point, to trace the growth in our knowledge of vitamin A more or less as a series of successive events, referring to a central theme. In dealing with further developments, however, it will be necessary to touch upon so many different topics that this procedure can no longer be followed It will only be possible to indicate the various fields of interest, which will be discussed in detail in subsequent chapters, without following any very logical order.

The pathology of avitaminosis A.

A considerable advance in the study of vitamin A deficiency was made in 1925 by Wolbach and Howe[1] After extensive research on the lesions sustained by rats they concluded that the fundamental abnormality was a substitution of stratified keratinising epithelium for normal epithelium The well-known xerophthalmia [illegible] ch also caused [illegible] An even wider [illegible][2] and Hughes, Lienhardt and Aubel[3] observed degeneration of the nerves in various animals, which proved that the site of action of the vitamin was not confined to epithelial tissues Further work by Mellanby[4] and Lane Moore[5, 6], moreover, brought bone formation into the picture, and indicated that constrictions of the nerves could result from skeletal abnormality. When we remember that deficiency of vitamin A also causes deterioration in dark adaptation it is clear that the range of lesions produced is remarkably wide. It can hardly be expected, however, that all the possible forms of injury will often be concentrated in the same individual

The physiology and biochemistry of vitamin A

Through the brilliant experiments of Wald[7] and of Morton[8] a fairly complete picture is now available of the mode of action of vitamin A in the retina Thus we know that it is reversibly oxidised by an enzyme system to its aldehyde, which combines with protein to form the photosensitive pigment rhodopsin, or visual purple

In most animals, however, the vitamin A present in the retina is negli-

References p 28

gible in quantity in comparison with the main store which is concentrated in the liver[9] The metabolic role of the vitamin in this organ is as yet unknown but certain other information is available Thus we know that the hepatic tissues contain the vitamin predominantly in esterified form and that by a delicate mechanism which is not as yet fully understood a more or less constant lower level of the vitamin in the form of the free alcohol is maintained in the blood plasma[10, 11]

Many investigations have been made on the effect of the richness of the diet in vitamin A and carotene on the extent of stores of vitamin A laid down in the liver Most animals under normal conditions of nutrition accumulate reserves which would ensure their survival for long periods on a deficient diet[12]

In studies of the physiology of vitamin A during reproduction however Dann[13] found that even when the maternal liver was rich in the vitamin the amounts transferred to the foetus were usually small The amounts of vitamin secreted in the milk were also small in relation to the reserves available in the maternal liver

The physiology of carotene Many of the problems relating to vitamin A have had to be studied in parallel on carotene and the other provitamins Moreover since different species of animals vary widely in the presence or absence of carotenoids in their blood and tissues it cannot be assumed that conclusions reached for one species will necessarily be applicable to another

Examination of the faeces in many animals has shown that carotene is absorbed much less efficiently than vitamin A In man and the cow even this inefficient absorption allows the plasma and fat to become strongly yellow In many other animals however the work of Deuel and others has shown that the fraction of the carotene intake which is absorbed is converted into vitamin A in the intestines[14, 15, 16] This finding must replace the early conclusion of Moore[17] that the conversion normally takes place in the liver Experiments on dogs[18] however have shown that this organ may also convert carotene if the pigment is injected by the portal vein

The differences between the carotene and vitamin A in their ease of absorption from the intestines have complicated studies on the effect of various agents on their metabolism Thus the administration of medicinal paraffin has been found to decrease the absorption of carotene, but to have little effect on that of vitamin A (Chap 16)

Interrelationship between vitamin A and other factors The interactions between vitamin A and sex hormones (Chaps 35 36) thyroxine (Chap 37) the adrenal hormones (Chap 20) and various other factors have received considerable attention In several instances these

factors have been found to have different effects on vitamin A and carotene.

Since the vitamin and its precursors are very liable to oxidation, trials of antioxidants to increase their stability were made soon after Drummond's unfortunate experience with carotene dissolved in ethyl oleate. For stabilising test solutions hydroquinone proved satisfactory. In animal tissues

in the intestinal tract

Vitamin A in the human subject.

In addition to observation on the effects of vitamin A deficiency, as observed clinically in the form of xerophthalmia and defective dark adaptation, numerous experimental studies have been made on the levels of the vitamin present in human blood and tissues In several investigations volunteers have been restricted to diets specially designed to be deficient in the vitamin.

During the period when the antimony trichloride method for estimating vitamin A was applied by means of the Lovibond Tintometer the levels of vitamin in the blood plasma were too low for satisfactory measurement, and most workers preferred to collect data on specimens of liver. This procedure had the disadvantage that estimations could only be made on dead subjects, and the normal range had to be deduced from cases of accidental death Large scale investigations were carried out by Wolff[21] and Moore[22] on livers taken at autopsy from both normal and diseased subjects Normal values were found to vary widely, but usually appeared adequate to have sufficed the requirements of the individual for a considerable period In some diseases, however, very low ranges were found Thus the average for chronic nephritis was only about one tenth of the normal average

The application of photo electric apparatus for the estimation of vitamin A by Dann and Evelyn[23] allowed the use of the antimony trichloride reaction with much greater precision, and marked a very real advance Kimble[24] soon followed with a minor classic in which she described the application of the photo-electric method to blood plasma Numerous papers on the estimations of vitamin in blood from normal and diseased subjects, with and without doses of vitamin A, have since been published As a result the range for carotene and vitamin A in the blood of healthy subjects are well known. In regard to disease it has been established, *inter alia*, that fever causes a

others have shown that normal urine, as might be expected, contains no

References p 28

vitamin A but that in some diseases such as pneumonia and chronic nephritis considerable amounts of the vitamin may be lost in the urine

Several investigations on the restriction of volunteers to deficient diets have been made of which the most extensive was carried out in England during the second world war (Chap 29) From the results of these trials it has been possible to estimate roughly the allowances of vitamin A and carotene which are necessary to prevent deficiency

Vitamin A in animal husbandry

Investigations of vitamin A in farm animals may be dated back to Palmer s experiments with chickens on a diet free from carotenoids (Chap 1) Some years later Hart Steenbock Lepkovsky and Halpin [26] demonstrated clearly in experiments with growing chicks that contrary to the statement by Sugiura and Benedict [27] birds require vitamin A Many papers have followed the pathological effects of vitamin A deficiency in birds and on the exact requirements necessary for successful poultry rearing and egg production

In regard to cattle early observations were made by Drummond and his colleagues on the influence of seasonal changes in diet on the vitamin A content of milk [28] and a voluminous literature has accumulated on this topic and on the high concentrations of the vitamin found in colostrum The lesions incurred in vitamin A deficiency have been studied by Lane Moore (Chap 26) and others Vitamin A deficiency under farming conditions has been observed in animals kept at pasture during severe droughts [29] or stall fed upon diets low in carotene [30] Research on vitamin A in bovines has therefore followed three main lines of interest Firstly the vitamin A requirements of the growing or adult animal have been studied secondly the transference of vitamin A from the cow to the calf and thirdly the variations in the vitamin A content of the milk which influence its value as human food

Drummond was also prominent in early work on the vitamin A requirements of pigs [31] Later Dunlop [32] obtained clear evidence of deficiency characterised by paralysis in pigs given practical rations which were not suspected of being defective A valuable contribution to our knowledge of the general effects of vitamin A deficiency was made by Hale [33] who observed various congenital abnormalities in the newborn offspring of sows maintained on a diet deficient in vitamin A during the first part of pregnancy Detailed studies of the corresponding lesions in rats were made later by Warkany [34]

The vitamin A requirements of sheep were investigated by Guilbert Miller and Hughes [35] by means of tests for adequate night vision More recently Australian workers have studied the same problems particularly in relation to variations in the intake of carotene caused by seasonal and climatic changes [36] [37]

Commercial developments. Cod-liver oil was used for the treatment of ailing, undeveloped or rachitic children, or as a safeguard against winter ills in children or adults, for many years before the existence of vitamins A and D had been demonstrated. The discovery of these vitamins allowed the quality of fish-liver oil to be judged on more scientific grounds than mere taste and appearance, and the desire of manufacturers and pharmacists to obtain the best prices for the most potent oils must have done much to stimulate the development of accurate methods of estimation. As the result of patient study over a number of years Morton and others have brought the estimation of the vitamin by ultra-violet spectroscopy to a high degree of perfection, and have devised procedures which allow its application to oils having strong absorption due to constituents other than the vitamin[38]. While our knowledge of the vitamin A requirements of human subjects, at different ages and in different conditions, can only be roughly guessed within limits of $\pm$ 50–100 %, we may calibrate our doses, and settle our financial transactions, with an accuracy of $\pm 2\frac{1}{2}$ %.

Large commercial developments have followed the discovery of the superiority of halibut-liver oil, and of many other fish-liver oils, over cod-liver oil. Markets have been found for oils of high potency and quality in pharmacy, while oils of lower standard have been used for poultry feeding and veterinary purposes Highly potent concentrates have been prepared by molecular distillation, and synthetic preparations of the pure vitamin are now available in substantial quantities

In Britain the fortification of certain brands of margarine with vitamins A and D, with the intention of making them equal to butter in nutritive value, was commenced on a voluntary basis over 20 years ago Compulsory fortification followed during the second world war, and has continued ever since Much of the vitamin A used for this purpose may be obtained from the liver of the whale, which also provides the blubber oil which is another of the main raw materials used in the manufacture of margarine

In the production of pharmaceutical and veterinary preparations of preformed vitamin A, and in the manufacture of vitaminised margarine, millions of pounds and dollars have been involved Carotene has been of relatively little commercial importance in pharmacy, but has received financial recognition as a component of animal fodders. Thus the price of artificially dried grass has been determined largely, and sometimes entirely, by its carotene content Carotene, obtained in this case from red-palm oil, has also been used as a supplement to preformed vitamin A in the fortification of margarine.

Some outstanding problems At the time of writing our knowledge of the pure chemistry of vitamin A appears to have outstripped our knowledge of its physiology and biochemistry.

References p 28

*It is true that the role of the small fraction of the body's content of vitamin A which is located in the retina has been clearly explained in relation to the special mechanism of dark adaptation (Chap 22) In regard to the general system, however, our ignorance is still profound Thus we realise that a balance must exist between the vitamin A reserve in the liver and the concentrations in the blood and other tissues, but are unable to explain either its mechanism or its purpose The remarkable influence of sex and adrenal hormones on the relative concentrations of vitamin in the liver and kidneys at least suggest a starting point for investigation on these puzzling points [39, 40]

We also know that in deficiency of vitamin A lesions are developed in sites, such as the mucous membranes and bones, in which the vitamin cannot readily be detected by chemical means even in the normally nourished animal Are the metabolic processes of these tissues influenced by concentrations of the vitamin which are too low for estimation, or does the vitamin exercise its activity either in changed form or by promoting the formation of some necessary metabolite in another part of the body? When the vitamin is used up, as indicated by decreased liver reserves, what are the products of its metabolism?

Perhaps the problem of the greatest practical importance, however, is to decide what benefit, if any, is derived from a high intake of vitamin A, sufficient to allow the accumulation of substantial liver reserves Thus normal wild rats, like normal humans, have considerable amounts of vitamin A in their livers Rats kept in captivity, however, may survive for indefinite periods and sucessfully reproduce, on allowances of the vitamin which are too low to allow storage Is it possible that the animals with substantial reserves may be better able than those without to resist physical and metabolic stresses? Experiments in France by Meunier have suggested that the resistance of rats to at least one form of stress, the necessity to detoxicate poisons such as sodium benzoate and bromobenzene, may be raised by considerably increasing the vitamin A allowance [41]

It is hoped, as one of the purposes of this book, to draw together from their scattered sources the various items of information which may have some bearing on these pressing problems

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PART II

THE ESTIMATION OF VITAMIN A AND ITS PROVITAMINS

CHAPTER 5

Biological Methods

General Introduction

Before studying the chemistry and physiology of vitamin A and of the pro vitamins from which it is derived we must understand the methods by which they are estimated There are indeed so many methods and they have been applied in such a multiplicity of modifications that a fully detailed account would both occupy excessive space and disturb the balance of our book

It is important however that the reader should appreciate the various kinds of problems that arise in vitamin A estimations and how they must be solved by the judicious choice of the technique appropriate in each particu lar case CHEMICAL AND PHYSICAL methods are complicated by the necessity of estimating two distinct groups of compounds headed by vitamin A_1 and β carotene respectively In each group further difficulties arise from the ex istence of congeners which vary both in their chemical properties and in the intensity of their biological activity The results of BIOLOGICAL TESTS may depend upon the basal diet chosen which may influence the relative activi ties of the preformed vitamin and its provitamins

There is therefore no universal method which can be relied upon to give a correct answer about the vitamin contents of any material The choice of the analyst will usually be made between biological spectrophotometric and chemical methods which may each be applied in different ways For certain purposes such as studying the distribution of the vitamin in tissues observ ation of the vitamin s FLUORESCENCE under ultraviolet irradiation provides a fourth method which is however little more than qualitative

For estimating preformed vitamin A in rich sources or a known pro vitamin which can be readily freed from other carotenoid pigments spectro photometric methods are by far the most accurate Skilful workers using good instruments may usually take readings accurate to $\pm 2\ 5\ \%$ Even higher accuracy is possible with the best apparatus used with special care When the vitamin or its provitamins are present in weak sources however the success of spectrophotometric methods will depend upon whether the

References p 39

vitamin can be separated, by preliminary stages of concentration from other substances which have absorption spectra overlapping with that of the vitamins Thus it may well be possible to make replicated readings on the final concentrate which agree closely among themselves, but this consistency will be useless if we cannot decide how much of the observed absorption is due to the vitamin When the vitamin is present in such a low concentration that its absorption makes only a minor and uncertain contribution to the final absorption of the concentrate, therefore, it usually becomes necessary to confirm the spectroscopic finding's by biological tests

Intermediate between the spectrophotometric and biological methods we may place the chemical methods, using this description to denote techniques which depend on the development of a colour on treatment with a reagent, rather than on the measurement of natural pre existing absorption As so defined chemical methods are only of practical value for estimating preformed vitamin A. The readings are usually rather less accurate than in spectrophotometric procedures, but it is often easier to avoid complications through the presence of contaminants when weak sources of the vitamin have to be examined

Biological Methods

Rat growth tests The most commonly used method for estimating either vitamin A or its provitamins by biological means depends on measurements of the rate of growth in rats when they are given graded doses of the substance under investigation (Fig 1) Young rats are taken at weaning from mothers which have received no rich source of vitamin A during the suckling period, and are given a diet deficient in the vitamin The diet used at the Dunn Nutritional Laboratory, Cambridge, consists of casein (vitamin free) 20 parts, sucrose 60, arachis oil 15, dried brewer s yeast 10, and mineral mixture 5 parts, with supplements of vitamins D, E and K After about 4–5 weeks the animals cease to grow, and often develop xerophthalmia Graded doses of the substance to be tested must then be given promptly to some animals, while others must be given graded doses of either the international preparation of β carotene or of the preparation of vitamin A acetate which has been more recently adopted Carotene is to be used when the substance to be given is of vegetable origin, otherwise the vitamin A standard is used

The increases in weight of the animals are recorded after a fixed period of 3–5 weeks, and "growth response curves" relating the average weight increases for groups of rats to the doses of the unknown and the standard are constructed Statistical methods, of which an excellent account has been

given by Coward [1], are then used to calculate the activity of the unknown in International Units. With the carotene standard the unit is fixed as 0·6 μg, and with vitamin A as 0·3 μg of the free alcohol Most workers agree with the official conclusion that under the conditions most commonly pertaining in biological tests these two units have virtually the same activity

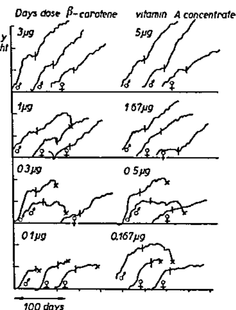


Fig 1 An early comparison by the author [9] of β carotene and a vitamin A concentrate ($E^{1\%}_{1\,cm}$ at 328 mμ = 950) in rat growth tests At all levels of dosing about the same responses were given by β carotene or by 1·68 times its weight of the impure vitamin A concentrate Dosing was started at the points indicated by vertical markings across the growth curves and was in some instances unduly delayed The tests could also be criticised on modern standards for the inadequate number of rats in each group and for the inclusion of slowly growing females along with the more fast growing males The cross at the end of certain curves is the conventional indication that the animal died

It is really quite easy to decide qualitatively by biological tests whether a material has vitamin A activity or not Accurate quantitative estimations, however, are not only difficult but also laborious and expensive Gridgeman [2] has calculated that when equal numbers of the male and female rats are used to test two doses each of the unknown material and the standard it is possible to obtain results which are within 77 to 130 % of the true value in 19 experiments out of 20 by using no less than 80 animals Accuracy may be slightly increased by using only male rats which grow faster than females, or by arranging rats from the same litter equally between different groups It has also been claimed that diets which include components such as meat meal or coconut meal, which presumably contribute unidentified nutrients, promote more rapid growth for a given dosage of vitamin A, and hence allow increased accuracy The evidence for this view, however, is not very convincing

In contrast to the factors which increase accuracy there are may technical imperfections and accidents which decrease accuracy If during the suckling period young rats can obtain access to food rich in vitamin A or carotene, such as liver, cod liver oil or carrots which are meant for their mother, they may fail to stop growing even if kept on a deficient diet for several months The few days before weaning during which they are learning to eat solid food allows the accumulation of substantial reserves of the vitamin The presence

References p 39

of such heavy reserves of course, will cause a cancellation of the whole test, but if only small reserves have been acquired the cessation of growth or 'running out", of the rats may only be postponed for 1–2 weeks At the higher body weights at which the animals will now be dosed, however, the growth responses on dosing may be more sluggish, and the accuracy of the estimation correspondingly reduced

Another major source of error affects even rats which have not accumulated significant reserves of the vitamin, and which have therefore "run out' promptly If the investigator is not confident that growth has stopped, or if for any other reason he delays dosing for more than a few days, his rats may decrease considerably in weight, and may contract intercurrent infections, such as pneumonia or cystitis (Chap 25) The diseased animal may then prove incurable and eventually die, or may make a slow recovery during which the rate of growth is not necessarily related to the dose of vitamin which is being given

Further complications arise in the calculation of results Should data for dead animals be omitted from the calculations? Our first decision, perhaps, is that they should be omitted But we may soon have to face an anomalous situation in which rats that barely survive through the test period will depress the average for the group, while those which actually die will not

For accuracy in biological tests for vitamin A, therefore, the resources of a large animal house must be placed at the disposal of a whole-time specialist He will take care that his young rats are obtained from mothers given a uniform diet, which will enable him to predict within a few days when any batch of rats will become deficient and stop growing He will give carefully calibrated doses to his animals by methods which ensure that the whole dose is eaten whether it is dropped into the mouth in liquid form or given mixed with the diet or on a palette in solid form If after sufficient experience he feels confident that his experimental conditions invariably cause "running out" within a certain period he may decide to carry out his tests on a preventive rather than a curative basis as recommended by Richards and Simpson[3] In any case he will not allow any of his animals to become seriously ill

Cure of xerophthalmia Many workers have taken the ability to cure xerophthalmia as good confirmatory evidence that a material under investigation contains vitamin A The abnormality has received less attention as the basis of quantitative estimations but a technique has been described by Coward[1] Rats deficient in the vitamin are taken at a uniform stage after the eyelids have become sore, but before the eyeballs are infected Groups are then given graded doses of the material to be tested or of the standard, and the times necessary to cure xerophthalmia

are averaged for each group With increasing doses of vitamin A the times necessary for cures should tend to become shorter

Cure of vaginal cornification When rats are deficient in vitamin A the vaginal lining does not undergo its usual changes according to the oestrous cycle but becomes fixed at one particular stage in which it is dry and cornified (Chap 36) This condition can readily be diagnosed by taking smears from the vagina with a spatula or glass rod Microscopic examination then reveals the presence of the thin square flakes which are typical of the condition If such rats are dosed with vitamin A the flakes disappear and smears taken at intervals show the successive pictures including epithelial cells and leucocytes which are typical of the other stages of the oestrous cycle (Fig 2)

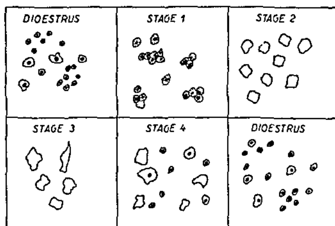


Fig 2 Vaginal smears at various stages in the oestrus cycle of the rat as described by Long and Evans[10] *Dioestrus interval* This constitutes about half the cycle Stringy mucus is seen with numerous leucocytes and small irregularly shaped single epithelial cells are also present *Stage 1 of Oestrus* The leucocytes disappear and give way to large numbers of small round nucleated epithelial cells of uniform size and appearance often in sheets This stage lasts for 12 hours *Stage 2* The epithelial cells are suddenly replaced by large thin non nucleated scale like elements (cornified or keratinised cells) At the beginning of this stage the female will accept coitus *Stage 3* This is an exaggeration of Stage 2 The rat will no longer accept coitus Stages 2 and 3 together last for 30 hours *Stage 4* Leucocytes begin to appear among the cornified cells which finally disappear Before all have gone however single epithelial cells reappear to usher in the dioestrous pause This stage usually lasts 6 hours giving an average of 4 6 days for the whole cycle In vitamin A deficiency the vaginal smear becomes fixed in Stage 2 or 3 The effect of dosing with the vitamin is to restore the normal cycle This action is plainly indicated by the reappearance of leucocytes in the vaginal smear

Baumann and Steenbock[4] suggested that these changes might form the basis of a method of estimating the vitamin with the advantage that adult rats could be used repeatedly to test different substances In confirmation of these hopes Moll *et al*[5] obtained sufficient accuracy with the new method

References p 39

at least to provide a useful check, specific for vitamin A, on the results of growth tests Further refinements were made by Coward, Cambden and Lee[6] who related the magnitude of single doses of cod liver oil with both the time taken for the flakes to disappear and the time which elapsed before they reappeared permanently in the smear Pugsley, Wills and Crondall[7] used ovariectomised rats, and obtained responses in the vaginal smear which varied logarithmically with the dose of vitamin

Prevention of nerve degeneration Another interesting method, devised by Coetzee[8] is based on the power of vitamin A to prevent nerve degeneration Young rats were taken from mothers which had been given a diet deficient in vitamin A during the last week of lactation and were kept upon the same diet, with graded doses of the material under investigation or of the international standard, for 5–6 weeks after weaning The degree of protection of the central nervous system was then examined histologically by the Marchi technique A high degree of accuracy was claimed but the method has not yet come into general use

THE IMPORTANCE AND DEFECTS OF BIOLOGICAL TESTS

Final arbitration Besides being superior to the spectrophotometric and chemical methods for estimating the vitamin A activity of very weak sources the biological methods are important as a 'final court of appeal'

The cases which have to be decided would appear to fall into two main groups In the first group we have to decide upon the activity of materials which do not show the spectroscopic and chemical criteria of the accepted forms of vitamin A but which for one reason or another are suspected of having biological activity All the natural and artificial congeners of vitamin A must have passed through this group when they were first discovered There are moreover, certain materials (Chap 23) which have been reported to contain vitamin A in a form which cannot be detected by physical or chemical methods For testing congeners and disguised forms of activity, therefore the biological method remains indispensible

The second group for which biological tests are still required includes materials in which the vitamin or its provitamins can be detected by spectrophotometric or chemical methods, but in which there are reasons for suspecting that the information given by these methods is misleading as an indication of the actual biological activity Raw carrots may be very rich in carotene, but will the animal be able to absorb the provitamin from such an indigestible medium? A stale fish liver oil may still have intense absorption at 325 mμ, but will its high degree of oxidation allow its vitamin A to be ab

sorbed and utilised by the animal without loss? Biological tests will take account of all such factors Not only will they integrate the contribution of all the congeners of vitamin A and carotene which may be present, but they will allow for the effect of the other constituents of the foodstuffs on their absorption and utilisation by the animal

Lack of discrimination It must be realised, of course, that there is an important converse to this process of integration. Biological tests do not provide the detailed information which is made available by the spectrophotometric or chemical methods Thus there is no discrimination between preformed vitamin A and its provitamins, or between the actual amounts that are present and the factors affecting their utilisation A manufacturer may want to know how much carotene his carrots contain as a basis for the chemical extraction of the pigment The efficiency of its absorption by the rat may not interest him, he may even comment that biological tests on rats will in any case have little bearing on the ability of his human customers to digest the same material A biochemist studying the conversion of carotene to vitamin A in a diseased patient will require to know the relative proportions that are present, and will be little interested in the result of laborious biological tests to find their combined activity

It is clear, therefore, that while biological tests are still essential for research on vitamin A, and for certain routine purposes they are decidedly limited in their scope Although they remain the foundation stone upon which our whole knowledge of vitamin A has been built they provide no answer to questions requiring discrimination, even in so simple a matter as distinguishing between vitamin A and its provitamins

Details of alternative basal diets and of vitamin A units and standards, are given in the appendix

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CHAPTER 6

Spectrophotometric Methods

PROVITAMINS

Estimations of provitamins are made exclusively by measurement of their yellow colour which is due to absorption in the region of 460 mμ The final reading of absorption is easy, but both the complete extraction of provitamins and their separation from other yellow pigments may be difficult Further complications may arise when it is necessary to discriminate between provitamins having different degrees of biological activity (see Chap 15)

Extraction Numerous methods for extracting carotenoids from food stuffs have been described Some depend on the digestion of the material with aqueous or alcoholic potash followed by the extraction of carotenoids by ether [1, 2] by acetone and ether [3] or by ethyl alcohol and light petroleum [4, 5, 6, 7, 8, 9] Lease and Mitchell [10] found however that when alkali is applied to vegetables rich in carbohydrates resins may be formed which prevent the full extraction of carotene Methods which do not employ alkali in the first stage are therefore more widely applicable Different workers have recommended direct extractions of the foodstuff with hot mixed light petroleum and acetone [11, 12] hot diacetone followed by light petroleum [13] and hot ethanol followed by hot light petroleum [14] Others have preferred prolonged soaking with cold acetone [15, 16] or macerating or grinding with various solvents [17, 18, 19, 20, 21] Booth [22] pointed out the objections to alkali heat and prolonged standing with solvents He made his first extraction by grinding the material in the cold with quartz powder and a mixture of acetone and light petroleum

Separations of provitamins After obtaining the crude extract the next step is to separate the provitamins from xanthophylls chlorophylls and other pigments which will interfere with the final spectroscopic measurements Many methods have been devised which depend essentially on making up the extract in 90% methanol or diacetone and extracting the provitamins with light petroleum In recent years however most workers have found that it is more convenient to separate the lightly adsorbed provitamins from more strongly adsorbed impurities on an

adsorption column Dicalcium phosphate, bone ash, and anhydrous sodium carbonate have been used successfully for this purpose Booth [22] has pointed out that the ideal adsorbent must be selective, stable and permeable to the passage of fluid, but that it must not cause destruction of the unstable provitamins He recommends the use of a suitable grade of alumina stabilised by the addition of anhydrous sodium sulphate (See Appendix)

Differentiation between the various provitamins

In extracts made without saponification from many foodstuffs and biological materials β carotene is the main yellow pigment which is obtained after purification, either by preferential solubility in light petroleum or by chromatography In such materials errors caused by the lower biological activity of the small amounts of α carotene and other provitamins which are present can be neglected and whole yellow colour can be conveniently estimated as β carotene Other materials, however, must be given special attention Some plants, and notably *Physalis*, contain esters of xanthophyll which closely resemble the provitamins in their solubility properties and in their chromatographic behaviour Before analysis these esters must be hydrolysed Maize contains a hydroxylated provitamin, cryptoxanthin (Chap 8), which is more strongly adsorbed than the carotenes Red palm oil contains substantial amounts of α carotene Tomatoes contain the inactive pigment lycopene, which resembles the carotenes in being preferentially soluble in light petroleum In certain marine tissues β carotene is only a minor constituent of the provitamin fraction Modified methods, usually based on chromatographic refinements, must therefore be worked out to meet the individual requirements for special materials

Spectrophotometric measurements

Various forms of apparatus have proved satisfactory for the final measurement of yellow colour With modern spectrophotometers, such as the Beckman and Unicam the use of a standard solution of carotene is unnecessary, since it suffices to take a reading at the wave length of maximum absorption and calculate the concentration of β carotene from its known extinction coefficient Since the extinction coefficient, and position of maximum absorption, vary according to the solvent it is necessary, of course, to base calculations on the extinction coefficient of carotene in the particular solvent used in the estimation

When simpler apparatus is preferred, for reasons of convenience or expense, colour filters may be used The Zeiss step photometer depends on this principle and has been used extensively. Photoelectric absorptiometers of simple type may be used in conjunction with standard curve based on measurements on pure β carotene with the same instruments Less exact readings may be made by means the standard graded glasses of the Lovibond Tinto-

References p 50

meter, or even by comparison with an aqueous solution of potassium dichromate, or with a solution of bixin in light petroleum [23] in colorimeters of the plunger type

The values obtained by any of these methods may be expressed as μg or mg of the provitamin in question Values for β-carotene may also be given in terms of the 0 6 μg international unit, and the amounts of the other provitamins may be similarly expressed with appropriate reductions for their lower biological activity In combining values for provitamins and preformed vitamin A in a foodstuff or diet the provitamin units are sometimes reduced to compensate for their incomplete absorption (Chap 30)

VITAMIN A

The problem of employing the intense ultraviolet absorption band of preformed vitamin A at 325 mμ for its quantitative estimation has attracted numerous investigators Special mention must be made, however, of the contributions in this field by Morton He participated in the first communication on the subject, except for isolated observations in Japan, about 25 years ago [24] and has remained a leading authority ever since The adoption of ultraviolet spectrophotometry as the main method for calibrating medicinal preparations and fortified margarine has given it great official and financial importance Long years of effort and ingenuity have therefore been willingly spent in attempting to overcome the many difficulties which stand in the way of its general application to all sorts of materials

Early developments As early as 1931 Coward, Dyer, Morton and Gaddum [25] reported an extensive investigation in which readings made by ultraviolet spectrophotometry and by the chemical method with antimony trichloride on a series of 11 cod-liver oils, and two concentrates, were compared with the results of biological tests The spectrophotometric readings were found to be more consistent than the chemical readings Soon afterwards a further paper [26] reaffirmed the reliability of the absorption at 328 mμ as a measure of the vitamin

At about this time the spectrophotometric method attracted the interest of official bodies concerned with the standardisation of vitamins, which included in Britain the Vitamin A Sub Committee of the Medical Research Council. This Committee had been largely responsible for proposing the adoption of β carotene as the International Standard As a step towards the official acceptance of the spectrophotometric method it became necessary to find a means of converting extinction coefficients, which measured the intensity of absorption of various sources, into international units per g It was hoped that when suitable precautions were applied the necessary conversion

factor C F would be a constant In this event the extinction coefficient of a 1 % solution of the material to be tested measured in a cell 1 cm thick could be related to the biological activity by a simple equation

$$E^{1\%}_{1\,cm} \text{ at } 328\ m\mu \times C\,F = \text{i u per g}$$

Old fashioned spectrophotometers worked on the principle of recording numerous twin ultraviolet spectra on photographic plates In the path of one spectra was placed a solution of the material containing the vitamin while in the path of the other there was an adjustable slit Curves were then obtained by matching the points of equal density on each pair of spectra and thereby finding the widths of the slit openings which were necessary at each wavelength to match the removal of light from the other spectrum due to absorption by the vitamin Skilled workers could duplicate readings with an accuracy of $\pm$ 5 % which at least was a great advance on the degree of accuracy obtainable by biological tests It was more difficult however to decide how much of the absorption at 328 mμ was due to vitamin A and how much to the glycerides in the fat and to other substances contributing irrelevant background absorption The importance of the irrelevant absorption moreover would vary in different oils In rich sources of the vitamin such as *halibut liver oil (Fig 3) it would make* only a small contribution to the total absorption but in weaker sources such as cod liver oil the contribution might be considerable (Fig 4) An obvious step therefore was to remove the glycerides by saponification The procedure adopted of course had to be chosen so as to avoid or at least minimise destruction of the vitamin The Vitamin A Sub Committee investigated techniques for the saponification of oils and a suitable standardised procedure was officially recommended [27]

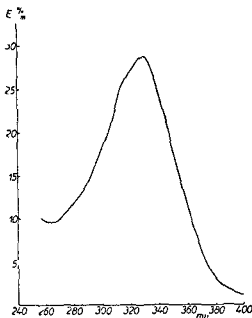


Fig 3 Spectroscopic estimation of vitamin A in halibut liver oil (by courtesy of R J Ward) The absorption spectrum measured on the whole oil has $E^{1\%}_{1\,cm}$ at 328 mμ = 28 7 The shape of the curve is only slightly different from that of pure vitamin A and the use of the old conversion factor of 1600 seems justified On this basis the oil contained 46000 i u per g which agrees well with the manufacturer s estimate The new conversion of 1900 applies only to spectroscopically pure sources of the vitamin (Unicam spectrophotometer)

In parallel with the spectrophotometric readings on the non saponifiable

References p 50

fractions of oils, comparisons of their biological activities with that of the standard β carotene were made by growth tests with rats On the basis of only a few such tests a conversion factor of 1600 between $E^{1\%}_{1\,cm}$ at 328 mμ and 1 u per gram of the oil or concentrate was adopted at the 2nd International Conference on Vitamin Standardisation in 1934[28].

Variations in the conversion factor

Criticisms of the conversion factor started soon after its official acceptance, and continued until the main standard was changed from carotene to pure vitamin A acetate in 1948 Thus Morgan, Edisbury and Morton [29], reasoning from the doubtful hypothesis, that low doses of carotene are quantitatively converted to vitamin A concluded that a conversion factor of more than 1100 was theoretically impossible In parallel biological and spectrophotometric estimations on a large series of oils and concentrates moreover, they found a wide variation in the ratio between the results obtained by the two methods. The biological activity per gram of pure vitamin A in each source, calculated on the prediction of pure vitamin having $E^{1\%}_{1\,cm}$ at 328 mμ = 1600, varied from 1 08 million for a mammalian concentrate up to 2 9 million for a specimen of cod liver oil For a time they entertained the idea that a highly active form of vitamin A having only weak spectroscopic absorption must be present in many concentrates

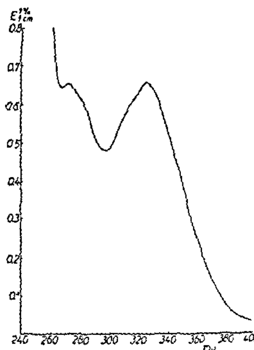


Fig 4 Attempted estimation of vitamin A in cod liver oil (by courtesy of R J Ward) The absorption spectrum measured on the whole oil has its maximum at 324 mμ with $E^{1\%}_{1\,cm}$ = 0 66 By the use of the conversion factor of 1600 the oil would be calculated to contain 1060 1 u per g But the shape of the absorption curve with the second maximum at 272 mμ, would make this calculation erroneous Saponification and probably other stages of purification would be necessary to allow an accurate estimation According to the makers the potency of the oil was only 700 1 u per g

The problem was later complicated by the detection in the mammalian sources, which for commercial reasons were not frankly described as whale-liver oils, of a substance which adsorbed at about 290 mμ, and which was presumably kitol (Chap 11), a derivative of vitamin A which is devoid of vitamin A activity [30] It was clear therefore not only that the conversion factor varied over a very wide range for different sources, but that the unsaponifiable matter in certain concentrates might contain substances other

than the vitamin having absorption spectra with their maxima close to 328 mμ.

Chevallier and Chabre [31] recognised that for the reliable spectrophotometric estimation of the vitamin it was essential that there should be a sharp absorption maximum at 328 mμ In whole oils they associated the presence of free acids, presumably indicative of oxidation, with increased general absorption.

Research therefore tended to develop in two different directions In the first place attempts were made to determine more precisely the conversion factor which could be applied in examining specimens of vitamin A having absorption spectra with sharp maxima at 328 mμ. Secondly methods were sought for eliminating inactive absorptive substances from concentrates before estimating the vitamin, or for making the appropriate deduction for their contribution to the absorption if they could not be removed

Determination of the conversion factor on sources having absorption spectra typical of vitamin A.

In order to supplement the slender evidence on which a provisional conversion factor of 1600 * had been calculated the British Vitamin A Sub-Committee planned an extensive collaborative investigation [32] Solutions of the standard β-carotene, of a specimen of halibut-liver oil, and of the unsaponifiable matter of the same oil were made up and checked spectrophotometrically by Morton They were then sent to eight British and one American laboratory so that their biological activities might be compared in growth tests upon rats. For the whole halibut-liver oil the mean for the conversion factor, weighted according to the numbers of rats used in the different laboratories, was worked out at the time as 1470, although after more careful statistical treatment this estimate was later revised to 1570 [33] For the unsaponifiable matter factors of only 1000–1200 were found, presumably because the concentrate had deteriorated during the dosing period

Similar comparisons were made in nine American laboratories between the standard carotene and a specimen of cod-liver oil which had been adopted by the U S Pharmacopoeia as a subsidiary standard The mean potency of the oil was 3000 i u per g, which indicated a conversion factor of about 2000. An unfortunate situation therefore arose in which vitamin A preparations lost about 20 % of their nominal activity in International Units when sent over the Atlantic in the eastward direction. The U.S.P. unit of vitamin A, which had been considered to be identical with the International Unit, must also have been 20 % smaller than had been supposed.

* Misunderstandings were inclined to arise for a time owing to both the conversion factor and the supposed extinction coefficient at 328 mμ of pure vitamin A happening to have the same value of 1600

Provoked by this anomaly the British group undertook a further investigation in which the U S P cod liver oil was compared with the standard carotene [33] The weighted mean for its activity was calculated as 2619 i u per g as against the mean of 3000 i u which had been found in American laboratories The Conversion Factor was 1820 which appeared to be significantly higher than the previous factor of 1570 found for halibut liver oil Until even more evidence had been collected however it was recommended that the official estimate of 1600 proposed in 1934 should be retained

Sources of error In the meantime various sources of error in the spectrophotometric method were extensively studied It was plain that unduly low biological potencies and hence low conversion factors were to be expected in unstable oils and concentrates in which the vitamin might deteriorate between the time of the spectrophotometric examination and the end of the prolonged biological tests Such deteriorations it was found could often be prevented either by the choice of a diluent oil which did not promote oxidation or by the addition of an antioxidant such as hydroquinone *Moreover if an oil intended to be employed as a standard was* first calibrated when fresh and then allowed to deteriorate before use all sources tested against it would appear to have unduly high activity British *opinion inclined to the view that such deterioration took place in the American* standard cod liver oil

Deterioration of the vitamin could also occur during the chemical processes necessary to separate the unsaponifiable fraction Such loss could readily result from the use of ether containing peroxides Embree [34] found that when the manipulations were carried out in ordinary glassware loss of the vitamin might be caused by exposure to light and the use of amber glassware was recommended Jones and Haines [35] found that during saponification larger amounts of vitamin A were lost from some types of oils such as halibut liver oils than from others such as shark liver oils

As might have been expected from experience with the carotenoids the absorption spectra of vitamin A concentrates were found to be affected considerably by the solvent used as diluent Thus for a series of oils and concentrates Smith Stern and Young [36] found mean extinction coefficient at 328 mμ in the proportion of 100 in ethanol 97 5 in cyclohexane 97 8 in hexane 107 5 in ether and 89 in chloroform Gillam and El Ridi [37] independently reached similar conclusions and further confirmation by other workers followed [38] [39] In making calculations from spectroscopic readings therefore the influence of the solvent had to be taken into account

Irrelevant absorption We may now turn to the second problem of estimating the vitamin in sources containing substances other than vitamin A which make a substantial contribution to the

absorption at 328 mμ When the gross extinction coefficients at this wavelength for such sources are multiplied by the usual conversion factor unduly high values are obtained

Chromatography has been used to remove interfering substances in many investigations[40 41 42 43 44] The adsorbent must be chosen with care, since undue strength may cause the production of coloured artifacts from the vitamin Glover Goodwin and Morton[45] found that chromatography on bone ash allowed *the removal of impurities without serious destruction of the vitamin*

Other methods have been based on the different principle of recording the absorption spectra of the material before and after the destruction of the vitamin by methods not calculated to influence the absorption due to other substances The absorption spectrum of the vitamin is then obtained by subtracting the second curve from the first Some workers used ultraviolet irradiation as the means of destruction[46 47] This method is open to the danger that the irradiation may cause spectroscopic changes other than those due to the destruction of the vitamin but it has been successfully applied to the examination of blood plasma[48 49] Awapara *et al*[50] described a similar procedure in which the vitamin was removed by adsorption on fullers earth rather than destroyed by irradiation

Objections to β carotene as the international standard

While these investigations were in progress criticisms of the use of β carotene as a standard continued It is certainly true that the *differences in the conversion factors used in America and Britain had caused* great inconvenience but many of the main difficulties involved in deducing the biological potencies of oils from spectrophotometric data were quite unrelated to this point Many workers pointed out however, that most tests of therapeutic preparations had to be made on sources of preformed vitamin A and that the use of the provitamin as the standard only introduced an unnecessary complication Moreover according to Gridgeman[51] and Brunius[52] there was ample evidence that the relative efficiencies of utilisation of carotene and preformed vitamin A could be influenced significantly by the character of the basal diet When vitamin A became readily available in pure form therefore the time seemed ripe for its replacing carotene as the main standard

An early comparison of crystalline vitamin A β naphthoate with the international standard carotene had already been made in 1943 by the British Vitamin A Sub Committee A conversion factor of 1770 was found which was significantly higher than the accepted factor of 1600 In 1946 Morton and Stubbs[53] studied the absorption spectrum of pure vitamin A acetate by means of a Beckman photoelectric spectrophotometer which allowed much

References p 50

more accurate readings than could be made by the photographic method

Vitamin A acetate as the standard

According to Oser[54] the U S Pharmacopoeial Revision Committee took the lead in recommending vitamin A acetate as the standard in 1948 This action may perhaps have been precipitated by evidence of instability in the third of a series of standard cod liver oils Although the Committee had not formally accepted the spectrophotometric method for estimating the vitamin A a conversion factor of 1894 was recommended The standard was dissolved in an oil which would ensure its stability Ellenberger Guerrant and Chilcote[55] found that the new standard remained stable during routine use in biological tests

Soon afterwards the suggestions of the U S P were accepted and amplified by the Expert Committee on Biological Standardisation of the World Health Organisation[56] Pure crystalline all *trans* vitamin A acetate $C_{22}H_{32}O_2$ was defined as having m p 57 8–59 0° C (Corr) and $E^{1\%}_{1\,cm}$ at 325 mμ = 1525 in *iso*propanol It was not stipulated whether the standard should be natural or synthetic in origin It was to be issued in solution in vegetable oil in such concentration that 1 mg of the solution contained 0 344 μg of the acetate which corresponds with 0 3 μg of vitamin A alcohol A review of extensive biological tests indicated that this amount was biologically equivalent to the old unit of 0 6 μg of β carotene On this basis the conversion factor for the acetate was calculated as 1900 For vitamin A alcohol with $E^{1\%}_{1\,cm}$ at 325 mμ — 1750 the conversion factor was also 1900 The old β carotene was to be retained for estimations on sources containing provitamins

Correction for irrelevant absorption

The W H O Subcommittee emphasised that the new conversion factor was only applicable to highly potent sources which had absorption curves almost identical in position and shape with that of the pure vitamin Thus it was laid down that the absorption maximum should lie between 325 and 328 mμ Between 310 and 350 mμ moreover the extinction coefficients for the source to be tested had to conform within limits of $\pm$ 2 % to the values calculated from the maximum extinction coefficient on the assumption that all the absorption was due to vitamin A It was pointed out that for the many sources which did not attain this high standard of purity the old conversion factor of 1600 had been more appropriate

In order to allow the use of the new conversion factor to a wider range of materials attention was turned to a procedure for correcting for irrelevant absorption which had been devised by Morton and Stubbs[57] This method depends essentially on taking readings on the specimen under investigation at the position of the absorption maximum for pure vitamin and at two other wavelengths one on each side of the maximum at which pure vitamin A has absorption equal to $^6/_7$ of the absorption at the maximum position Cor

rection for irrelevant absorption can then be made by geometrical and algebraical methods (see Appendix)

The validity and general applicability of this procedure have been studied by several investigators [58, 59, 60] Adamson *et al.* [61] reviewed the results of estimations on a series of oils carried out in 7 laboratories, and concluded that the use of the correction procedure greatly increased the difficulty in obtaining consistent results Thus duplicate readings on the gross absorption for any oil had a limit of error of $\pm$ 2 % for $P = 0\ 05$, but after the correction procedure had been applied the corresponding limit was $\pm$ 15 % Possibly this criticism, apart from commercial transactions, may be less serious than it seems, particularly in the examination of sources with a high proportion of irrelevant absorption It is better to have a reading within $\pm$ 15 % of the true activity of a source than within $\pm$ 2 % of a fictitious value which may diverge widely from the true activity It must be realised, however, that in the procedure of Morton and Stubbs two out of the three readings have to be taken at wavelengths where the absorption curve usually slopes steeply At such points the readings must tend to be less accurate than at the flat top of the curve

Additional findings Subsequent to the acceptance by the W.H O Sub-Committee of the absorption spectrum of Morton and Stubbs as characteristic of vitamin A acetate, Chatain and Debodard [62] reported values for a specimen, prepared from the natural vitamin, which appeared to indicate a slightly higher degree of purity The extinction coefficients in various solvents were 1–2% higher than the accepted figures at the maximum, and 4–5 % lower, at certain other points

Attention was redirected to the effect of solvent on the absorption spectrum in a large collaborative investigation by Boldingh *et al* [63] and the influence of esterification was also studied The following conversion factors were recommended

Solvent	*Vitamin A alcohol*	*Vitamin A acetate*
*iso*Propanol	1825	1906
Ethanol	1825	1850
Cyclohexane	1906	1906
Light petroleum (40–60°)	1825	1825

Cama and Morton [64] have reported the surprising finding that cod-liver oils may contain appreciable amounts of vitamin A_2 and have suggested methods for estimating the contribution which it makes to the total absorption Analytical difficulties introduced by the presence of neovitamin A, and other *cis* isomers, have not yet been fully investigated (Chap 11).

References p 50

General conclusions The foregoing account of the spectrophotometric estimation of the vitamin cannot pretend to be complete It may indicate, however the extreme complexity of a highly specialised field which has absorbed the energies of many skilled workers over a period of about 20 years General agreement on the principles underlying methods for estimating the vitamin in many of its sources should now be reached without undue delay, but doubtless certain other materials will continue to give difficulty

Those who are cynically inclined might perhaps comment that all the intensive work since the 1934 Conference on Vitamin Standardisation has only led to the changing of the conversion factor from 1600 to 1900 The factor of 1600 chosen so shrewdly on the slender evidence available 20 years ago could be applied without correction to the most highly potent sources of the vitamin then available For all but the purest modern sources the new factor of 1900 must now be applied in conjunction with a correction procedure This adjustment has still to be proved fully reliable and often has the effect of reducing the conversion factor to about its previous value of 1600

It might also be considered that attempts have been made to force the accuracy of the spectrophotometric method beyond the limits that have been reached in other directions in research on vitamin A Our knowledge of the human requirement for the vitamin must allow an uncertainty of at least $\pm 50\%$ What can be the point, therefore, in straining after a much higher degree of accuracy in calibrating vitamin A concentrates? The answer is probably to be found mainly in the desirability of maintaining fixed standards of potency in pharmaceutical and commercial preparations and so allowing the corresponding financial transactions to be carried out on an equitable basis The research worker with a general interest in vitamin A must be grateful that this motive has resulted in a much more thorough exploration of one corner of this field than might otherwise have been expected

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CHAPTER 7

Colour Reactions and Fluorescence

The importance of colour reactions in the early development of our knowledge of vitamin A and its provitamins has already been indicated (Chap. 2). We must now consider the conditions which must be satisfied if reliable estimations are to be made by colorimetric methods, and the techniques which have been evolved for this purpose.

The first difficulty to be faced is that colours with antimony trichloride, or other reagents used for estimating vitamin A (Table 2), are produced not only by the vitamin, but by numerous other substances Thus the carotenoids as a class, whether they are provitamins or not, are all chromogenic. Since they often accompany the vitamin in its natural sources they have to be removed before the antimony trichloride is applied, or alternatively a correction has to be made for their contribution to the total blue colour. Other substances which produce colours, such as certain oxidation products of cholesterol [1, 2, 3], have not been detected in natural sources of the vitamin, and hence have only to be kept in mind as possible simulators of the colour reactions of the vitamin when unusual materials have to be tested.

A second difficulty arises from the inhibitory action which several classes of substances exert in preventing the full development of colour reactions of vitamin A In the preceding chapter we have seen that spuriously high values are obtained in testing sources in which the vitamin makes only a partial contribution to the total ultraviolet absorption In the colour reactions, however, this tendency is reversed. With many cod-liver oils the intensity of the blue colour may be doubled by the removal of glycerides by saponification, while with butter fat the increase may be 10-fold

Thirdly we have to overcome the technical difficulty that the colour produced with antimony trichloride, the most popular reagent for estimating vitamin A, is not permanent With concentrated sources of the vitamin the extent of fading within the first few minutes after adding the reagent can usually be neglected, but with impure sources there may be complete fading within a few seconds The apparatus employed for measuring the blue colour must therefore be capable of rapid operation, particularly if weak sources of the vitamin are being examined.

References p 65

TABLE 2

COLOUR REACTIONS USED FOR THE DETECTION OR ESTIMATION OF VITAMIN A

Reagent	*Reference*	*Colour produced*	*Permanent* (P) *or transient* (T)
Sulphuric acid	Chap 2	Purple	T
Fullers earth	(1)	Blue	T
Arsenic trichloride	(10)	Blue	T
Antimony trichloride	Chap 2	Blue	T
Guaiacol phenol and $HClO_4$	(34)	Purple	P
Glycerol dichlorhydrin	(37)	Purple	P

In spite of difficulties, however, the antimony trichloride method, and other colour reactions, have certain compensatory advantages Thus they are not only applicable to weaker sources of the vitamin than can be readily examined by ultraviolet spectrophotometry, but can be applied with less labour in manipulation and with much less expensive apparatus For these reasons the antimony trichloride reaction has been used far more extensively than any other method in providing the data on which our knowledge of the physiology and biochemistry of vitamin A has been based It seems fair to consider ultraviolet spectrophotometry as the favourite method of the pharmacist for estimating the vitamin, and the antimony trichloride reaction as the favourite method of the biochemist

Preliminary Manipulations

Extraction procedures Various techniques have been employed for the extraction of the vitamin from its sources and the choice between them is usually guided by the nature of the material to be examined Very rich oily sources, such as halibut liver oil will usually give reliable readings after simple dilution with chloroform Cod liver oils, as already mentioned require saponification The oil is heated with alcoholic potash until the mixture is homogeneous, water is then added and the vitamin is extracted with ether A popular method for liver, and other animal tissues, depends on the preliminary digestion of the tissues with aqueous alkali, followed by the extraction of the vitamin by ether in the presence of alcohol [4] Alternatively the tissues may be extracted in the cold by grinding with ether and an abrasive such as sand or quartz powder Yet another method is to grind the material to a solid mass with anhydrous sodium sulphate, and then extract it with ether in a Soxhlet apparatus

Some materials present special difficulty Casein, for example must usually be extracted with hot ethyl alcohol before colour tests can be applied successfully [5] Blood is best treated by adding alcohol and extracting with light

petroleum [6], digestion with alkali is also satisfactory for this material if sufficiently prolonged, but inadequate digestion causes low results To some extent well established methods of extraction are applied to certain materials as a routine without full assurance that interfering substances have been eliminated Thus extractions of blood without saponification of the extract, or extractions of liver by alkaline digestion of the tissues without complete saponification of the fat, may both be suspected in certain circumstances of giving low results Data obtained in surveys by such well known methods, however, have the advantage of being directly comparable with the results of previous work, which have often been accumulated over a long period of years

Dehydration, evaporation, purification Before the application of reagent the extracts obtained by the different methods must be further treated with the general purpose of obtaining a clean dry fatty residue, or unsaponifiable fraction, ready for solution in chloroform This may prove difficult if emulsions have been formed between the ether and aqueous layer during the extractions of alkaline digestions or the products of saponification, but with practice emulsification may usually be avoided When the solvent is ether it may be dehydrated before evaporation by adding anhydrous sodium sulphate and filtering, or by filtering through a layer of this material The extracts in light petroleum obtained in extracting blood, however do not require treating with sodium sulphate

When a clean dry residue has been obtained, preferably by evaporating under reduced pressure on a warm waterbath, it is sometimes necessary to resort to further steps for the removal of interfering substances For this purpose the residue is usually dissolved in light petroleum and fractionated by chromatography Vitamin A alcohol is adsorbed much more strongly than its esters Thus in the common problem of separating the vitamin from accompanying carotenoids the esters run near to carotene, and the free alcohol is difficult to separate from xanthophylls Narod and Verhagen [7] recommended chromatography on magnesia mixed with Celite as a means of separating vitamin A and its esters from carotenoids in extracts of animal rations which had been fortified by adding the vitamin

THE ANTIMONY TRICHLORIDE METHOD

Technique of colour measurement For the production of colour the final extract is dissolved in a measured volume of chloroform To a small volume of this solution 0 5 ml or less contained in a standardised test tube or optical cell a drop of acetic anhydride is first added, which is followed by 2 ml of the antimony trichloride reagent * According to

* The reverse order however, may sometimes be preferable for details see Appendix

References p 65

the original instructions this reagent is made by dissolving antimony trichloride in chloroform at the concentration of 30 % weight in volume The reagent is best added by means of an automatic dispenser. In Britain a suitable model has been designed by British Drug Houses Ltd , but several other modifications have been described [8, 9]

The production of colour If vitamin A is present in the extract a bright blue colour is formed immediately the reagent is added The shade is very bright, and is perhaps best described as "peacock blue In the spectroscope a sharp absorption band is readily seen at about 620 mμ Deviations from the normal shade do not necessarily mean that the vitamin is absent, but must at least arouse suspicion Thus substances present in the extract may either influence the colour formed by the vitamin or change the shade seen by contributing colours of their own The carotenoids for example, react with antimony trichloride more slowly than the vitamin and usually go through a green stage lasting several seconds, before producing duller blue shades Presumably the yellow of unchanged carotenoid is mixed for a time with the blue produced by pigment which has reacted with the reagent The oxidation products of vitamin A often contain a substance which gives a red colour. Extracts which give a purple colour instead of the typical bright blue must therefore arouse suspicion that an active source has undergone deterioration

If the addition of the reagent to the extract causes cloudiness the most probable explanation is that the extract was wet, and that the drop of acetic anhydride which was added was inadequate to fulfil its intended purpose of combining with any remaining traces of moisture Water causes the conversion of antimony trichloride to the white, insoluble oxychloride When colour is matched by visual methods, e g in the Lovibond Tintometer, the occurrence of clouding is readily perceived, and the test is rejected With modern photoelectric apparatus in which the vitamin is added to a cell al ready in position, however, it is much easier for clouding to escape detection To avoid the recording of unduly high readings, therefore, the cells should be inspected on removal from the apparatus in order to make sure that no clouding has occurred The formation of a film of oxychloride on the optical surface must be avoided by cleaning the cell frequently between tests Concentrated hydrochloric acid is useful for this purpose

The intramolecular changes which presumably give rise to the product of colour are discussed elsewhere (Chap 11)

The measurement of colour Numerous methods of measurement, some crude and some refined have been recommended for measuring the intensity of the antimony trichloride reaction In their description of the arsenic trichloride reaction Rosenheim and Drum

mond [10] recommended matching the blue colour produced against a standard solution of methylene blue mixed with crystal violet Later Oshima and Itaya [11] devised a similar procedure for the antimony trichloride reaction, but with graded standard solutions made up with copper sulphate mixed with cobalt nitrate instead of the dyestuffs Leong [12] suggested an even rougher method based on the matching of colour against a piece of blue paper which he supplied with his communication Such crude methods may still be of some service in enabling agricultural or medical workers, who do not specialise in vitamin research to make rough tests on liver oils without special apparatus

Attempts to refine methods depending on the use of dyestuffs as standards, however, attracted little interest after Rosenheim and Schuster [13] had introduced a modification of the Lovibond Tintometer as a suitable instrument for the rapid matching of the fading blue colour produced by the vitamin It was arranged so that the blue solution could be seen through the eyepiece in one section of an illuminated field while graded blue glasses could be quickly introduced to cover another part of the field Differences in shade were adjusted by the simultaneous introduction of yellow, or sometimes red glasses In the hands of most workers the Tintometer was only capable of giving results with an accuracy of about $\pm$ 15 %

Concentrations had to be adjusted so that the reading was within the range 4–6, and most observers could discriminate only with difficulty between readings differing by 0 5 Large systematic errors between the readings of different observers were also troublesome The apparatus, however, had no optical or electrical parts to go wrong and any abnormality in colour production was at once apparent It can still be recommended for use when the results are expected to spread over a wide range e g surveys of the vitamin A reserves in human or animal livers

Much more precise readings were made at the wavelength of maximum absorption by Morton and his colleagues [14] by means of a visual spectrophotometer Van Eekelen *et al* [15] recommended the Zeiss step photometer, an accurate visual instrument which employs a series of spectral filters for the measurement of different colours This apparatus was later used extensively by several European workers, including With [16]

For estimating small quantities of the vitamin in impure extracts however, both the Lovibond Tintometer and the more refined visual methods were too slow for the satisfactory measurement of the full intensity of colour before fading had occurred This was a serious handicap for example, in attempts to estimate the small concentrations of the vitamin which are carried in blood plasma The successful application by Dann and Evelyn [17] of the photoelectric colorimeter therefore greatly widened the field of research in which

References p 65

the antimony trichloride reaction could be used Various types of photoelectric apparatus have subsequently been applied Some have followed the Evelyn model in using filters, but others, such as the Beckman and Unicam have diffraction systems which allow measurements to be made at the exact wave length of maximum absorption In the author's laboratory a simple single cell instrument, consisting of a light source, filter, barrier type photocell and galvanometer, has proved satisfactory The constancy of its calibration has been checked frequently by recordings on Lovibond glasses

Two other methods, which have not come into general use, deserve mention for their ingenuity Hoch [18] carried out the antimony trichloride reaction on a micro scale in a small tube, which he photographed in red light together with a number of other tubes containing graded concentrations of cuprammonium sulphate Urban, Milder and Carruthers [19] devised a system in which a beam of light, after passage through the reaction cell, was divided into two parts These were passed for a fixed period through filters absorbing at 620 mμ and 589 mμ respectively, and then fell upon photocells, which were connected to condensers The charges accumulated were considered to measure vitamin A and carotene respectively

Units and calculations The colour reactions for vitamin A were first applied to cod-liver oils, and Rosenheim and Drummond [10] found that a convenient intensity of colour could usually be obtained by adding one drop of oil of about 20 mm^3 to 1 ml of arsenic trichloride With the introduction of the Lovibond Tintometer and antimony trichloride reagent the same proportions were retained, and it was customary to add 40 mm^3 of the oil, or of a diluted solution, to 2 ml of the reagent The Lovibond reading observed, corrected if dilution had been necessary, was recorded as the Colour Value of the oil

Colour values indicated concentrations rather than quantities of vitamin A, and the diverse methods adopted by different workers in calculating quantities of vitamin from colorimetric data led to great confusion over a long period For use in his work on the conversion of carotene to vitamin A the author found it essential to devise a colorimetric unit, rather than a colour value The unit later known as B U (Moore) was taken as the amount of blue colour contained in 1 ml of solution viewed in a layer 1 cm thick, when giving a Lovibond reading of 1 blue This ' blue unit" was in due time calculated to be about 0 6 of the international unit

The introduction of photoelectric methods had been preceded by the isolation of the vitamin in virtually pure form With those instruments which were calibrated to measure extinction coefficients, therefore, it was possible to calculate the concentration of vitamin directly from a knowledge of the extinction coefficient of the pure substance (see Appendix) With other instru-

ments the pure vitamin could be used for the construction of individual calibration curves In many modern communications results are now quoted in μg of vitamin A Since the international unit is 0 3 μg data expressed in μg may be converted to i u by multiplying by 3 3

Inhibitors We may recall that cod liver oil manufacturers at one time considered that the production of a strong purple colour on treatment of an oil with sulphuric acid was only an indication of objectionable oxidation (Chap 2) Their aim therefore was to produce oils giving as little colour as possible Evidence for this view moreover was still forthcoming after an association between the colour reaction and vitamin A had been accepted Thus Hawk [20] found that when he left bottles of cod liver oil open to air and exposed to light for about two weeks the colour produced with antimony trichloride was intensified Since the vitamin was known to be destroyed by oxidation he concluded that the antimony trichloride reaction was not an accurate measure of vitamin A potency

At about the same time earlier observations by Mittelmann were reported by Bezssonoff [21] When cod livers were sealed into evacuated tins and then autoclaved at 120° C a pleasant tasting oil was obtained which gave no colour with antimony trichloride If the tins were opened and examined again 10 days later however the oil was now found to give a positive reaction with antimony trichloride and at the same time to have acquired an unpleasant fishy odour Although Lovern Creed and Morton [22] failed to confirm that oils prepared by Mittelmann s method gave no colour at all with antimony trichloride they substantiated his finding that the colour produced was increased during storage The same effect was seen in oils made by steaming the livers Colour production could be increased artificially moreover by treating the oils with ozone

Heilbron Gillam and Morton [23] had noticed that the colour reaction with antimony trichloride of most oils containing vitamin A was characterised by two absorption bands with their maxima at 572 mμ and 606 mμ respectively In fresh oils the 572 mμ band predominated but as the oils became stale the absorption at 606 mμ increased For a time it appeared that the substance responsible for the 606 mμ band might be derived from a non chromogenic precursor through oxidation The 572 mμ band appeared to be more closely associated with the ultraviolet maximum at 328 mμ and was considered to give a more reliable indication of biological activity

Emmerie Van Eekelen and Wolff [24] however put forward another view When substances such as indole skatole or furan were added to the unsaponifiable matter of liver oils the colour produced with antimony trichloride was changed from blue to purple Spectroscopic studies indicated moreover that this change was due to the inhibition of the absorption maximum seen in the

References p 65

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610 mμ region The band at 572 mμ was relatively unaffected Morton[25] confirmed this finding using 7 methyl-indole as an inhibitor

These experiments proved clearly that inhibitors could affect the ability of vitamin A to produce its normal colour with antimony trichloride Indole derivatives however, were presumably to be expected in oils made from putrid livers rather than in samples made under sterile conditions from fresh livers If the increase of the blue colour in stale cod liver oil was to be ascribed to the destruction of inhibitors, therefore, their chemical nature was obviously quite different from those already investigated By extracting the fatty acids of cod liver oil with about 80 % sulphuric acid Emmerie[26] separated a small yield of an unsaturated acid, $C_{21}H_{36}O_3$ which had 4–5 times the inhibitory power of indole

Booth, Kon Dann and Moore[27] showed that the low values obtained when the antimony trichloride test was applied to butter fat could be explained by the presence of an inhibitor, which affected added vitamin A no less than the vitamin originally present in the butter The amount of inhibitor was greater in summer than in winter butter, and thus went parallel with the concentration of the more highly unsaturated fatty acids

Besides being inhibited the antimony trichloride reaction of vitamin A may sometimes be masked by colours produced by fats which are used as diluents Most vegetable fats produce pale brown colours, which increase on standing and which are given much more intensely by oxidised than by fresh oils Fresh coconut oil, which is almost completely saturated, has very low inhibitory power and the antimony trichloride test may be applied directly to vitamin A dissolved in this medium

Correction for carotenoids The extinction coefficient of the blue colours given by various carotenoids and by vitamin A in the antimony trichloride reaction are given in Table 3 It will be seen that the colours produced by the carotenoids are all much less intense than that produced by the vitamin

In estimating vitamin A when it is mixed with carotenoids the concentration of carotenoids must first be measured by the yellow colour The blue colour which the pigments contribute in the antimony trichloride reaction must then be calculated and subtracted from the total colour Complications arise however from differences both in the speed of the development of the colours given by vitamin A and by the carotenoids and in the effects of other components of the extract In the author s experience some substances tend to increase the intensity of the blue colour given by carotenoids It is not very satisfactory therefore to make corrections on the basis of data obtained on pure specimens of the vitamin and carotenoids Before undertaking a series of estimations on any type of material a special correction

TABLE 3

SPECTROSCOPIC DATA BY GILLAM [48] ON THE NATURAL YELLOW COLOURS OF CERTAIN CAROTENOIDS AND ON THE BLUE COLOURS PRODUCED WITH ANTIMONY TRICHLORIDE

	Max	Yellow colour in $CHCl_3$ $E^{1\%}_{1\,cm}$	$SbCl_3$ reaction Absorption measured at	$E^{1\%}_{1\,cm}$	Colour
β Carotene	463	2200	585 *	420	Slate blue
Lycopene	480	2000	615	260	Blue
Lutein (ex nettles)	453	1800	615	360	Blue
Zeaxanthin	460	1500	615	370	Blue
Astacene (ex lobsters)	500	1900	590	1000	Violet blue
Fucoxanthin (ex *Fucus vesiculosus*)	458	1450	615	290	Blue
Vitamin A			620	5070	Blue

* The position chosen for measurement was not always the maximum For β carotene however the reading at 585 mμ was greater than at 615 mμ whereas for lycopene lutein and zeaxanthin the reading at 615 mμ was greater than at 585 mμ

curve should be made from measurements of the blue colours produced by the carotenoids concerned when present in extracts of the particular material In making this curve moreover the time after the addition of the antimony trichloride at which readings are taken must be appropriate for vitamin A This reading for the correction must not be delayed past the normal time for taking the vitamin A reading even if the colour produced by the carotenoids has not had time to reach its maximum

When vitamin A can be separated from the carotenoids efficiently by chromatography or other means the results obtained in this way will be both more accurate and more readily interpreted than results obtained by the correction procedure When routine estimations have to be made however the desire for accuracy has usually to be balanced against pressure of time In large series of estimations of vitamin A and carotenoids in blood the author has corrected the vitamin A values expressed in i u by subtracting one quarter of the carotenoids expressed as i u (0 6 μg units) Unfortunately when the same correction was applied to the blood of Jersey cows which often contains as much as 2000 i u of carotenoids per 100 ml as compared with only about 200 i u in humans the corrected values for preformed vitamin A were sometimes less than zero This experience will

References p 65

illustrate the difficulty of finding a correction factor which is generally applicable, and the necessity of separating vitamin A from mixtures in which carotenoids greatly predominate

Other Colour Reactions

Reactions with pyrocatechol and guaiacol The two main defects of the antimony trichloride reaction are the transience of the colour, and its production by carotenoids as well as by vitamin A In 1933 Rosenthal and Erdély [28] proposed a modification which was not open to these objections One ml of a chloroform solution containing the material to be tested was mixed with 1 ml of 0 5 % pyrocatechol in chloroform and 3 ml of the antimony trichloride reagent When this mixture was heated to 60° C for 1–2 minutes the presence of the vitamin was indicated by the appearance of a reddish-violet colour, characterised by absorption bands at 552 and 476 mμ Later guaiacol was found to be superior to pyrocatechol in giving an even more stable colour [29]

Andersen and Levine [30] confirmed that the red colour was not given by carotenoids, but found that it could be produced by heating the usual reaction mixture in the antimony trichloride test in absence of pyrocatechol or guaiacol Willstaet [31], Van Eekelen [32] and Balassa and Szanto [33] pointed out that although Rosenthal's reaction had the advantage of not being given by carotenoids it had the disadvantage of being given by cholesterol, which made it unsuitable for the examination of tissue extracts Van Eekelen also found that the colour continued to develop over a long period, which made it difficult to decide when the maximum had been reached

In a reaction of the same type, devised by Pacini and Taras [34] the solution containing the vitamin was made to give a stable purple colour by treatment with guaiacol, phenol and a solution of perchloric acid in chloroform

Fearon's reaction The mechanism of the reactions just described is obscure It might be thought that the red colour is produced by an oxidation product of the vitamin Since the polyhydric phenol does not appear to be essential for the production of colour it may possibly act by accelerating an oxidation which takes place more slowly in its absence

The use of a phenol, however, is reminiscent of Fearon's reaction [35], which was proposed some 30 years ago, for the estimation of the vitamin A red colour was developed when cod liver oils were treated with a solution of trichloro acetic acid in light petroleum in the presence of pyrogallol and an oxidising agent A critical investigation by Rosenheim and Webster [36], however, proved that this reaction may be given in the absence of the vitamin It appears, therefore, that the vitamin is no more necessary than the

phenol for producing some sort of red colour when fatty substances are treated with condensing agents According to Rosenheim and Webster the colour given by cod liver oil in Fearon s reaction was probably a condensation product between the phenol and aldehydic oxidation products of highly unsaturated fatty acids such as clupanodonic acid $C_{22}H_{34}O_2$

The glycerol 1 3 dichlorhydrin reaction

Although the method of Rosenthal and Erdely never became popular presumably on account of the criticism of Van Eekelen and others a rather similar method introduced by Sobel and Werbin[37] has been quite extensively used When solutions of vitamin A are treated with glycerol 1 3 dichlorhydrin which is also known as 1 3 dichloro 2 propanol an unstable blue colour is first formed with an absorption band at 625 mμ which is near the maximum seen with the antimony trichloride reagent This colour changes rapidly to a stable violet or purple with a maximum at about 550 mμ, which coincides with one of the bands seen in the reaction of Rosenthal and Erdely Carotene was found to give a green colour with the new reagent with a main maximum at 475 mμ and a subsidiary maximum at 625 mμ

Feinstein[38] advocated the addition of small quantities of hydrochloric acid to activate the glycerol 1 3 dichlorhydrin When the reagent was applied with this modification colorimetric measurements gave results which agreed well with data obtained by ultraviolet spectrophotometry Sobel and Snow[39] later confirmed the desirability of activating the reagent which they effected by distillation in the presence of antimony trichloride They found the new method satisfactory for examining blood serum Sobel and Werbin[40] applied the activated reagent to numerous specimens of fish liver oils and their concentrates and obtained values which averaged 4 per cent higher than those found by the antimony trichloride method and 26 per cent lower than those found by ultraviolet spectrophotometry

Penketh[41] agreed with Feinstein in finding hydrochloric acid an effective activating agent and also produced some activation with sulphuric acid In the experience of Allen and Fox[42] better activation is induced by hydrochloric acid and chlorosulphonic acid than by sulphuric acid

Further progress was made by Sobel and Rosenberg[43] in working out a micromodification of the glycerol 1 3 dichlorhydrin method suitable for estimating vitamin A and carotene with small quantities of milk When the reagent was applied to the unsaponifiable fraction of the milk fat accurate estimations of vitamin A could be made by measuring the absorption at 555 mμ by means of a Coleman universal spectrophotometer The estimation of carotene was made some minutes later on the same solution by reading the absorption at 800 mμ

Sobel and his colleagues are justified in their claims that the glycerol 1 3

References p 65

dichlorhydrin reagent is superior to antimony trichloride in giving a permanent colour and in allowing vitamin A and carotene to be estimated in the same solution Since the reaction is unaffected by small amounts of water the clouding and film formation which give trouble in the antimony trichloride reaction are avoided

The disadvantage of the glycerol 1 3 dichlorhydrin reaction are the lower intensity of the colour as compared with the antimony trichloride reaction and the large deduction which has to be made for carotene if the orthodox correction procedure is applied The method moreover is still open to the criticism that the vitamin probably undergoes more changes in producing the 555 mμ absorption band than in producing the 625 mμ band The possibility that the 555 mμ band may be given by biologically inactive oxidation products of the vitamin cannot therefore be ruled out Assurances are also required that cholesterol derivatives or at least those likely to occur in sources of vitamin A are incapable of contributing to the colour formed under the influence of glycerol 1 3 dichlorhydrin

A Partially Chemical Technique

The liver storage method

This chapter seems appropriate for mention of the liver storage method of Koch and Kaplan[44] which is partly biological and partly chemical Young rats are first made deficient in vitamin A They are then dosed for a few days with the material to be tested containing either the preformed vitamin or provitamins and are killed for the estimation of the vitamin A contents of their livers by the antimony trichloride reaction It is claimed that a constant threshold dose of the vitamin disappears but that any excess over this level is stored quantitatively in the liver

This ingenious method appears to make use of the rat instead of the chemical laboratory for the preliminary concentration of vitamin A from weak or impure sources It also gives the equivalent in preformed vitamin A of any provitamins which are present in the material to be tested We may suspect however that the doses administered must be carefully chosen if consistent results are to be obtained It would be interesting moreover to find out how closely results obtained by this method would coincide with the results of either biological or chemical tests applied separately

Fluorescence Under Ultraviolet Irradiation

Detection

In 1926 Peacock[45] made the first detailed study of the yellow fluorescence shown by cod liver oil under ultraviolet irradiation He found that both the yellow fluorescence and vitamin A activity were lost

in oil which had been exposed to intense white light In some way which still remains unexplained the fluorescence, but not the biological activity was partially restored when oil which had been exposed to light was kept for some months in the dark

Estimation Brocklesby and Rogers[46] suggested that the fluorescence might be made the basis for quantitative estimations Promising results were obtained when the vitamin was dissolved in benzene and titrated with maleic anhydride until the fluorescence was lost Alternatively aqueous suspensions of the sources were titrated with bromine in acetic acid or the fluorescence of emulsions made with glycerol was compared with standards Sobotka, Kann and Winternitz[47] suggested that vitamin A esters might be distinguished from the free alcohol by the 4 fold increase in their fluorescence after irradiation for a few minutes In spite of these interesting observations, however, no trustworthy method of estimating the vitamin by its fluorescence has yet been evolved The mechanism of the fluorescence, and the factors which effect its intensity remain obscure

As a qualitative indication of the presence and particularly of the location, of the vitamin the fluorescence has had more useful applications Thus in margarine factories the fluorescence of fats was at one time used for routine checks on their adequate fortification with the vitamin In histological studies knowledge has been gained about the distribution of the vitamin in liver, and other tissues by examining their fluorescence under the microscope (Chap 19) Finally fluorescence has been found useful for locating the position of the vitamin on chromatographic adsorption columns

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PART III

THE CHEMISTRY OF VITAMIN A AND ITS PROVITAMINS AND CONGENERS

CHAPTER 8

The Occurrence, Characterisation and Chemistry of the Provitamins

In his masterly treatise on the carotenoids [1] Professor Paul Karrer states that about 80 of these pigments are known to occur naturally, and that for about 35 of them the chemical structure has been fully elucidated Only 10 natural carotenoids however, are listed as being capable of serving as provitamins A (Table 4)

For human nutrition β carotene is certainly the most important provitamin In most common foods it greatly exceeds the other provitamins in concentration The presence in its molecule of two intact β ionone rings, moreover, confers a biological activity at least twice as intense as those of the other provitamins α Carotene accompanies β carotene in many of its sources, in amounts of up to 30 %, while the proportion of γ carotene is usually less than 1 % Of the other provitamins the cryptoxanthin of yellow maize has undoubtedly the greatest importance for humans and land animals

Provitamins such as myxoxanthin and echinenone may of course have vital roles in the provision of vitamin A for marine animals, but they have little immediate importance for land animals There is still some uncertainty, moreover, about their chemical constitution Goodwin and Taha [21] have indeed advanced strong evidence that echinenone and the plant pigments aphanin and myxoxanthin may all be identical

Apart from the provitamins certain other carotenoid pigments, which often accompany them in their sources, and which complicate procedures for their estimation, require mention in a book on vitamin A. Among these pigments are the familiar xanthophyll of egg yolk and the tomato pigment lycopene A list of the more familiar "inactive" carotenoids is given in Table 5

The Isolation of Provitamins from Vegetable Sources

In green plant tissues the bluish green chlorophylls are invariably accompanied by yellow xanthophylls and carotenes, usually with the concentration of the xanthophylls about twice that of the carotenes On account of the presence of a hydroxyl group in each of their ionone rings the xanthophylls are much more readily soluble in alcohol than the carotenes and less soluble

References p 84

TABLE 4

NATURAL CAROTENOID PIGMENTS CAPABLE OF ACTING AS PROVITAMINS A

	Formula	Structure	Source	Reference
β-Carotene	$C_{40}H_{56}$	Two β-ionone rings	green vegetables, carrots	2, 3
α Carotene	$C_{40}H_{56}$	One β-ionone ring, one α ionone ring	accompanies β carotene Red palm oil	4, 5, 6, 7
γ Carotene	$C_{40}H_{56}$	One intact β-ionone ring, one opened β-ionone ring	various	8, 9
α Carotene epoxide	$C_{40}H_{56}O$	One oxygenated β-ionone ring 5, 6, one α-ionone ring	certain blossoms	10
Citroxanthin = mutatochrome	$C_{40}H_{56}O$	Two β ionone rings, with oxygen attached to one of them by a furanoid linkage 5, 8	orange peel	11
Cryptoxanthin	$C_{40}H_{55}OH$	Two β-ionone rings one hydroxylated 3	yellow maize, *Physalis*, *Carica papaya*	12, 13, 14
Myxoxanthin	$C_{40}H_{54}O$	One intact β-ionone ring, one β-ionone ring opened and oxidised to form a ketone	algae	15
Aphanin	$C_{40}H_{54}O$	Probably two β-ionone rings with a carbonyl group in one of them	*Aphanizomenon flos aquae*	16
Echinenone	$C_{40}H_{58}O$	Unknown, but possibly a ketone	sea urchins	17, 18
Torularhodin	$C_{36}H_{47}COOH$	Probably one intact β-ionone ring with the remains of another β-ionone ring which has been opened and oxidised to a carboxyl group	red yeast (*Torula rubra*)	19, 20

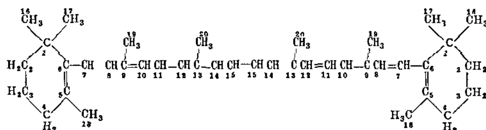
TABLE 5

SOME FAMILIAR CAROTENOID PIGMENTS WHICH ARE INCAPABLE OF ACTING AS PROVITAMINS

	Formula	*Structure*	*Sources*	*References*
Xanthophyll* or lutein	$C_{40}H_{54}(OH)_2$	α carotene with hydroxyls at 3 and 3′	green leaves egg yolk	2 22
Zeaxanthin	$C_{40}H_{54}(OH)_2$	β carotene with hydroxyls at 3 and 3′	maize *Physalis* egg yolk	23 13 24
Lycopene	$C_{40}H_{56}$	as β carotene but with the β ionone rings opened between 1 and 6 and 1′ and 6′	tomato	25
Astacene	$C_{40}H_{48}O_4$	β carotene with keto groups at 3 3′ and 4 4′	lobster shells, salmon	26 27
Astaxanthin **	$C_{40}H_{52}O_4$	β carotene with keto groups at 4 4′ and hydroxyls at 3 3′	lobster eggs	28

* Xanthophyll zeaxanthin and other hydroxylated carotenoids are often grouped together under the general name of xanthophylls

**At the time of writing the question that astaxanthin may have some degree of biological activity is under investigation (see Chap 23)



Numbering system for carotenoids Karrer s method of numbering the carbon atoms of the carotenoid molecule is shown above as applied to β carotene In describing products formed with the loss of part of the molecule the term apo is used and the double bond at which the oxidation occurred is counted from the end of the molecule Thus β apo 2-carotenal denotes an aldehyde formed by oxidation between carbon atoms 7′ and 8 In β apo 4 carotenal oxidation has occurred between carbon atoms 11′ and 12′ Other workers have sometimes related the term apo to the number of the last remaining carbon atom rather than to that of the double bond

References p 84

in light petroleum The well known "phase test", in which solutions of mixed carotenoids in light petroleum are shaken up with methanol containing a little water, is based upon this difference Xanthophylls, which pass into the lower methanolic layer are described as "hypophasic pigments", while the carotenes are "epiphasic" Cryptoxanthin, with its single hydroxy group is epiphasic with 85 or 90 % methanol, but becomes hypophasic with 95 % methanol Lycopene, containing no hydroxyl groups, accompanies the carotenes when the phase test is applied to tomato extracts For purposes of analysis, therefore, it must be removed from the provitamin fraction by other means (See Table 6 for lists of the more familiar epiphasic and hypo phasic pigments)

TABLE 6

THE PARTITION OF THE MORE FAMILIAR CAROTENOID PIGMENTS BETWEEN LIGHT PETROLEUM AND 90% METHANOL

Epiphasic	*Almost equally distributed between both layers*	*Hypophasic*
α- β and γ Carotenes	Cryptoxanthin	Xanthophyll
Lycopene		Zeaxanthin
α Carotene epoxide		Astacene
Mutatochrome		Astaxanthin
Esters of xanthophyll etc		

The presence of hydroxyl groups in the xanthophylls also facilitates their removal from solution by adsorbents One of the earliest achievements in chromatography, a technique which has now assumed great importance in both pure and biological chemistry, was the separation of the carotene and xanthophyll fractions, both from themselves and from chlorophylls (see Chap 6) Subsequent intensive studies have led to numerous refinements of the original crude procedure, particularly in the careful choice of suitable solvents and adsorbents, and substances which differ much less radically than in the presence or absence of hydroxyl groups may readily be separated To revert to the analysis of tomato extracts we may separate the open chained lycopene molecule from the ringed carotene molecules by taking advantage of the greater ease with which it is adsorbed Suitably activated alumina is chosen as the adsorbent, with light petroleum containing an appropriate amount of methanol as the solvent

In addition to being present in all green vegetable tissues carotenes may also be found in most yellow vegetable tissues Thus the carrot root, from which the carotenoid pigments as a class took their name, contains carotenes

in amounts which are directly indicated by the intensity of its yellow colour. Appearances may be deceptive, however, for intensely yellow flowers, such as marigolds, may owe almost all their colour to xanthophylls The yellow pigments in turnips, moreover, are misleading as a guide to the presence of provitamins

The presence in the carrot root of large amounts of carotenes unaccompanied by more than traces of other pigments makes this source the ideal starting point for the isolation of carotenes on the laboratory scale The ease with which the pigment crystallises, particularly on the addition of alcohol to its solution in solvents such as benzene, carbon disulphide or chloroform, further facilitates its isolation in crude form and doubtless explains the success of Wackenroder [29] in preparing the first specimen with the limited facilities which were available over 100 years ago

The Properties of β Carotene

Pure β carotene, $C_{40}H_{56}$ may be obtained from crude preparations by chromatography Thus Karrer and Walker [30] purified substantial amounts of crude carrot carotene by adsorption from light petroleum upon calcium hydroxide The α isomer is less readily adsorbed According to Karrer dark violet hexagonal prisms of β carotene are obtained from solutions in benzene on the addition of methanol, or red rhombic, almost quadratic crystals from light petroleum Most workers have reported melting points of 181–182° The symmetrical structure of the molecule gives rise to no optical activity Karrer has reported absorption maxima at 520 485 and 450 mμ with carbon disulphide as the solvent 497 and 466 mμ in chloroform, 483 5, 452 and 426 mμ in light petroleum and 477, 450 and 425 mμ in hexane In 1944 a careful comparison of two specimens of β carotene was made at the National Physical Laboratory England [31] One specimen had been prepared by Karrer ten years previously for use as an International Standard, and the second had been freshly made at Lever Bros , Port Sunlight, England, with a view to replenishment of supplies of standard when the first batch became exhausted From Figure 5 it will be seen that the absorption curves for the two specimens were almost identical The extinction coefficients at the wavelengths of maximum absorption, with benzene and cyclohexane as the solvents, were as follows

	Benzene		*Cyclohexane*	
	mμ max	$E^{1\%}_{1\,cm}$	mμ max	$E^{1\%}_{1\,cm}$
Karrer s specimen	464	2284	455	2456
Lever's specimen	465	2290	456	2441

References p 84

β-Carotene is readily soluble in carbon disulphide, chloroform and benzene, but evidence that it is less soluble than the α isomer may be found in the concentration of that form in the mother liquor during the crystallisation of impure specimens Ether and light petroleum are less efficient solvents, *n* hexane dissolves 109 mg of β-carotene per 100 ml at 0°. In ethanol and methanol the pigment is almost insoluble

For physiological reasons the solubility in natural fats may perhaps be of special interest Deep orange yellow solutions may certainly be obtained in all fats, but it must be realised that on a basis of weight the concentrations of pigment which can be held in solution are comparatively low Artificial solutions made up to contain more than about 1 mg per ml of oil cannot be relied upon to resist crystallisation when stored in a refrigerator This limited solubility, which may be contrasted with the virtual miscibility of preformed vitamin A in oils, introduces difficulty in experiments in which it is desired to administer large doses of β-carotene in ready available form to human subjects or animals

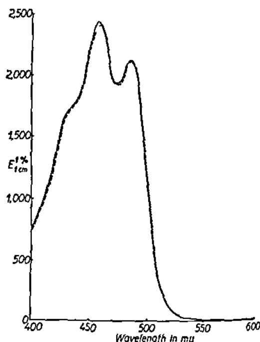
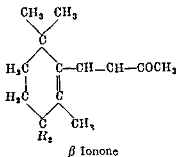
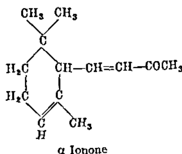


Fig 5 The absorption spectra in cyclohexane of two specimens of β carotene The plain curve was found for a specimen prepared by Karrer, and the broken curve for a specimen prepared by Lever Bros The curves are almost identical

β Carotene remains stable for years when it is stored *in vacuo* or in an atmosphere of inert gas In air at room temperature oxidation is soon indicated by the odour of β ionone (see Formula Group 1), which is considered by some



β Ionone



α Ionone

Formula Group 1 The ionones are liquids of high boiling point with a characteristic odour of violets In β carotene both ends of the molecule have the structure of β ionone *In α* carotene one end of the molecule has the β structure and the other the α structure

workers to be suggestive of violets The final product of oxidation is colourless, and at least part of the residue is insoluble in chloroform and other solvents which readily dissolve the intact pigment β Carotene is also unstable in solution in the presence of oxygen, and its destruction is increased in light Solutions in chloroform bleach very readily in sunlight In natural fats and synthetic fatty acid esters the stability of carotene varies greatly, but in general its destruction is hastened by the processes of autoxidation and retarded by the presence of antioxidants, such as hydroquinone

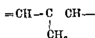
In general these remarks on stability may also be applied to other carotenoids

The Structure of β Carotene

The chemical structure of β carotene was deduced by Karrer and his co-workers[32 33 34], who started their investigation with a preliminary knowledge of the molecular formula of $C_{40}H_{56}$[(2)] and of the presence of 11 double bonds which could be saturated by hydrogenation[35] The intense yellow colour suggested that most of these unsaturated linkages must be co-ordinated in a conjugated system

In a study of the products formed by destructive oxidation with permanganate a number of dimethyl compounds were detected, including α, α dimethylglutaric acid, α, α dimethylsuccinic acid, dimethylmalonic acid and geronic acid (α α dimethyl δ acetyl valeric acid) Similar dimethyl oxidation products could be obtained from β ionone and from the yields which were obtained it was calculated that the β carotene molecule contains two β ionone ring systems (See Formula Group 2)

In addition to dimethyl compounds the oxidation products also contained acetic acid in an amount indicating four groups of the type



It was therefore concluded that the two β ionone rings are joined by a chain of 4 isoprene units with the evolution of the structural formula which has since been generally accepted Final proof of its validity has recently been obtained by the successful synthesis of the pigment by Karrer and Eugster[36]

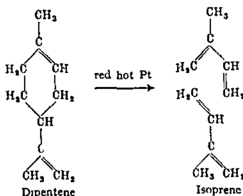
In Formula Group 3 the structural formula of β carotene is divided up to show how it is based on the skeletons of 8 isoprene molecules The formation of isoprene from dipentene is shown in Formula Group 4 The occurrence of the isoprenene skeleton in other molecules of biological importance will be discussed in connection with the chemistry of vitamin A (see Chap 9)

β Carotene → Geronic acid → α, α Dimethyl glutaric acid

→ α α Dimethyl succinic acid → Dimethyl malonic acid | *iso*Geronic acid from α carotene

Formula Group 2 The products obtained by the destructive oxidation of β carotene which gave Karrer and his colleagues evidence of the presence of β ionone ring The same products were obtained from each end of the molecule With α carotene the oxidation proceeds through geronic acid at one end of the molecule and through *iso*geronic acid at the other

Formula Group 3 The above structural formula of β carotene has been marked off to show how it contains the skeletons of 8 molecules of isoprene, C_5H_8 In the combining together of the isoprene units 8 hydrogen atoms have to be discarded and the double bonds have been extensively rearranged In regard to the spacing of the methyl groups in the central chain it will be noticed that 5 carbon atoms intervene between the 1st and 2nd and 3rd and 4th methyl groups but that the 2nd and 3rd methyl groups are divided by 6 carbon atoms This is due to the reversal of the asymmetric isoprene molecule which is orientated inwards from each ionone ring This point will be made clear by the two molecules of isoprene shown under the centre of the β carotene molecule



Formula Group 4 The production of isoprene an unsaturated hydrocarbon of b p 34° by passing the vapour of dipentene an optically inactive form of the limonene of orange peel pine needles etc over red hot platinum Isoprene may also be obtained by the destructive distillation of natural rubber

Chemical Reactions of β Carotene

Stability to alkali In common with most other carotenoid pigments β carotene is stable in alkali at least in the absence of oxygen With due precautions therefore the pigment may be recovered in tact after the saponification of fats with alcoholic potassium hydroxide

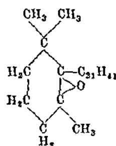
Colour reactions Again in common with most other carotenoids carotene produces colours with various reagents including sul phuric and nitric acids With antimony trichloride a blue colour is obtained as with vitamin A The reaction is less rapid however and the shade is less bright with its maximum at 590 mμ as against 620 mμ for vitamin A β Carotene also resembles vitamin A in giving a blue colour when it is adsorbed on acid fullers earth

Oxidation products Karrer and Jucker [37] studied a number of crystalline products formed by the oxidation of β carotene with monoperphthallic acid The first product to be formed was β carotene mono epoxide It will be seen from Formula Group 5 that it has an oxygen atom attached between two carbon atoms on the inner face of one of the β ionone rings This epoxide was found to be very sensitive to mineral acids which caused it either to revert to unoxidised β carotene or to pass into mutato chrome with the formation of a furanoid ring More severe oxidation with dilute chromic acid was found by Kuhn and Brockmann [38] to cause the opening of one of the β ionone rings with the formation of semi β carotenone By resorting to permanganate Karrer and his colleagues [39, 40] completely oxidised away one of the β ionone rings with the formation of an aldehyde

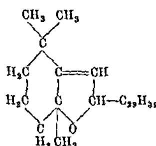
References p 84

Extensive investigations, in which Karrer again played a prominent part, indicated that most of these changes could occur at either one or both ends of the molecule It was possible, moreover, for two different changes to occur at different ends of the same molecule, with the formation of substances such as β-carotenone aldehyde A large number of different oxidation products have been formed in this way It will be noticed that furanoid ringed mutatochrome is listed among the naturally occurring provitamins in Table 4

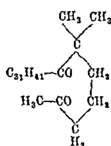
Discussion of oxidation as a step in the conversion of β carotene to vitamin A may be postponed until a later chapter (see Chap 17)



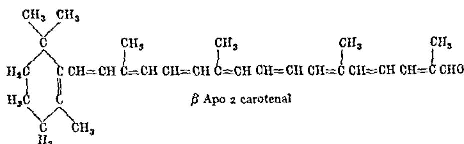
β Carotene epoxide



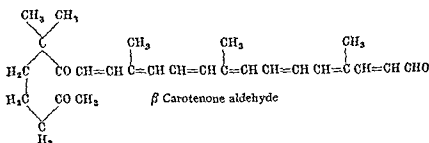
Mutatochrome, with furanoid ring



Semi β carotenone



β Apo 2 carotenal



β Carotenone aldehyde

Formula Group 5 Some products obtained by the mild oxidation of β carotene The groups $C_{31}H_{41}$ or $C_{29}H_{39}$ represent the remainder of the molecule arranged as in β carotene, with the β ionone ring still intact Of these oxidation products only β carotenone aldehyde must be devoid of biological activity, having lost both its β ionone rings *In deference to the usual conventions the unchanged part of the molecule is placed on the right in the first two compounds and on the left in the second two There is no real reason for this distinction*

Hydrogenation and dehydrogenation products. Karrer and Ruegger [41] obtained β dihydrocarotene, $C_{40}H_{58}$, by reducing β-carotene with aluminium amalgam It is interesting that the rearrangement of the double bonds of the central chain which results from the addition of the two hydrogen atoms causes a complete loss of biological activity. Dehydro-β carotene, $C_{40}H_{54}$ which was first described by other investigators as *iso*carotene, was shown by Karrer and Schwab [42] to be formed from β-carotene by the loss of two hydrogen atoms when it was first treated with iodine and then with thiosulphate acetone, mercury or finely divided silver. Again the redisposition of double bonds caused a complete loss of biological activity (see Formula Group 6)

β Carotene

β Dihydrocarotene

Dehydro β carotene (*iso*carotene)

Formula Group 6 The formation of biologically inactive derivatives from β carotene by the addition or removal of two atoms of hydrogen It will be seen that in each case the system of double bonds has been displaced with the appearance of a saturated linkage at the centre of the molecule

Cis trans isomerisation In 1935 Gillam and El Ridi [43] found that when β carotene was submitted to repeated chromatographic absorption it was divided up into two zones The upper zone consisted of the unchanged pigment, and the name pseudo α carotene was given

References p 84

to the pigments of the lower zone, presumably because it resembled α carotene in being less strongly held than the β isomer on the absorption column

As the result of further investigations the formation of the new pigment was ascribed to *cis-trans isomerism* According to Zechmeister and his colleagues [44] this form of isomerism, which depends on the parts of molecule on each side of double linkages being turned towards each other or away, occurs only at those double bonds in the central chain which are adjacent to methyl groups On this basis 16 isomers could presumably be formed, as against the 512 which could be formed if isomerism could take place in any of the 9 double bonds in the central chain

It has now been established that *cis trans* isomerism, which can occur in other carotenoids, may be induced by refluxing the pigment in a solvent, by illumination, by treatment with acids or iodine, or by melting the crystals [45-51]. The conversion between *cis* and *trans* forms of carotenoids is reversible, but the occurence of *cis* forms in natural sources is infrequent Zechmeister [52] considers that the main effects of *cis* isomerism are lowered melting point, increased solubility, reduced yellow colour with a shift in the maxima towards shorter wavelengths, and the appearance of pronounced "*cispeaks*" in the ultraviolet absorption spectrum The presence of *cis* isomers is perhaps most readily demonstrated by ultraviolet spectroscopy which reveals the presence of the characteristic maxima at wavelengths about 142 mμ shorter than the main maximum in the visible.

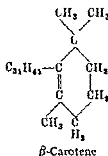
Discussion of the effect of *cis* isomerism on biological activity will be deferred until chapter 15

α- AND γ CAROTENES

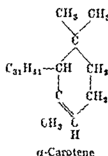
α Carotene, m p 187–188°, differs from the β isomer in having one β and one α-ionone ring as against two β-ionone rings [53] (see Formula Group 7) In all solvents it is more soluble than the β form, thus its solubility of 294 mg in 100 ml of hexane at 0° is nearly three times greater than that of the β carotene. It resembles the other carotenes, however, in being practically insoluble in methyl and ethyl alcohols Crystallisation from benzene and methanol gives violet prisms and clusters, and from light petroleum dark violet prisms or polygons In carbon disulphide the absorption maxima are at 509 and 477 mμ, and in hexane at 475, 445, 420 and 395 mμ The asymmetric molecule confers high optical activity, with a specific rotation of + 385° in light of 644 mμ [54]

The chemical properties of α-carotene generally resemble those of β carotene, but by the preservation of the α-ionone ring during oxidation a different set of products may be obtained Moreover with only one double bond in

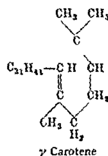
a ring system adjacent to the central chain it is possible for only mono epoxides to be formed By treatment with sodium ethoxide α carotene may be converted into the β isomer [55] Carotene from red palm oil may contain as much as 30 % of the α isomer [7, 8] (See Appendix)



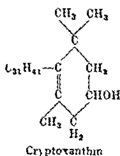
β-Carotene



α-Carotene



γ Carotene



Cryptoxanthin

Formula Group 7 In the above provitamins the group $C_{31}H_{41}$ in each case contains the β ionone ring which appears to be essential for biological activity This structure is repeated at the other end of the molecule in β carotene In α carotene the other end of the molecule is an α ionone ring in γ carotene an acyclic structure as in lycopene and in cryptoxanthin a hydroxylated β ionone ring as in zeaxanthin

γ Carotene was first separated by Kuhn and Brockmann [56] in a yield of 0 1 % from a mixture of crude carotene which also contained about 85 % of β-carotene and 15 % of the α form Its properties suggested that its structure must be intermediate between those of β carotene and lycopene Soon afterwards Winterstein [57] isolated the pigment as a major component of the carotenoids of the fruit of *Gonocaryum pyriforme* a plant which grows in the East Indies The presence of twelve double bonds in conjunction with an elementary formula $C_{40}H_{56}$ gave further support for a molecule with one cyclic end resembling carotene and one acyclic end resembling lycopene (see Formula Group 7) Although chemical evidence that the ionone ring is of the β form is not conclusive this inference may perhaps be drawn from the growth promoting activity of the pigment γ Carotene crystallises from benzene and methanol in microscopic red crystals m p 176 5–178° It has no optical activity In carbon disulphide its absorption bands are at 533 5 496 and 463 mμ and in hexane at 494 462 and 431 mμ

References p 84

"δ- and ζ-Carotenes"

In his extracts of *Gonocaryum* Winterstein also detected a new carotenoid making up 10 % of the total pigments, which he named δ carotene Reports followed of the detection of the same pigment in carrots [58] in certain micro-organisms [59, 60] and in high concentration in specially selected varieties of tomatoes [61, 62] According to Porter and Murphy [63] the absorption maxima in *iso*octane are at 280, 430, 456 and 488 mμ Probably "δ carotene" is a dihydro derivative of γ-carotene rather than a carotene isomer It has no vitamin A activity

The name ζ carotene was given by Strain and Manning [64] to a pigment with absorption maxima in light petroleum at 400 and 425 mμ which Strain [65] had previously separated from carrots Other workers confirmed the presence of such a pigment in carrots, maize and tomatoes [66, 67, 68] Studies by Nash, Quackenbush and Porter [69] have however suggested that "ζ-carotene" is actually an octahydrolycopene with an elementary formula of $C_{40}H_{64}$ This conclusion is in agreement with its inactivity as a source of vitamin A

The weight of evidence suggests, therefore, that the names of both δ and ζ-carotenes are unjustified and are misleading in suggesting that they should be included in the list of provitamins

Cryptoxanthin

This pigment, which differs from β-carotene in having a hydroxyl attached to one of its β-ionone rings (see Formula Group 7), was first isolated under the name of caricaxanthin from *Carica papaya* by Yamamoto and Tin [24] These workers reported an incorrect elementary formula of $C_{40}H_{56}O_2$ Later Kuhn and Grundmann [70] isolated the pigment under its present name while studying the carotenoids present in the berries and calyxes of *Physalis franchetti* and *alkekengi* In earlier work Kuhn and his colleagues [71, 72, 73] had already separated "physalien' the dipalmitate of zeaxanthin, from this source but without detecting the new mono-hydroxylated pigment The choice of a name meaning "hidden xanthophyll" is thus readily understandable

Cryptoxanthin, $C_{40}H_{56}O$, resembles zeaxanthin in being present in the *Physalis* in esterified form, and it may be isolated after saponification by a procedure based mainly on its being more readily soluble than zeaxanthin in light petroleum Crystallisation from a mixture of benzene and methanol gives lustrous prisms, which tenaciously retain some methanol, m p 169° In carbon disulphide, chloroform, benzene and pyridine the pigment is easily soluble, but it dissolves less readily in light petroleum, ethanol or methanol

As might be expected it is adsorbed in chromatography more readily than the carotenes but less readily than xanthophylls having two hydroxyl groups Its absorption maxima are at 519 483 and 452 mμ in carbon disulphide and at 484, 451 and 423 mμ in hexane Cryptoxanthin is effective as a provitamin A It can form a mono epoxide, devoid of biological activity, and also a di epoxide [74] *Cis-trans* isomers have also been described [75]

The isolation of cryptoxanthin from yellow maize was accomplished by Kuhn and Grundmann [76] in 1934 In most specimens of the cereal which were examined the concentration of cryptoxanthin amounted to about half that of zeaxanthin, and was at least 7 times greater than that of carotene

Artificial Provitamins

In his book Karrer [1] lists 15 derivatives obtained by chemical treatment from carotenoid pigments which have vitamin A activity These are β carotene mono-epoxide, $C_{40}H_{56}O$, β carotene di-epoxide $C_{40}H_{56}O_2$, luteochrome, with one epoxide and one furanoid oxygen grouping $C_{40}H_{56}O_2$, dihydroxy-β carotene, $C_{40}H_{56}O_2$, semi β-carotenone $C_{40}H_{56}O_2$, semi-β carotenone monoxime, anhydrosemi-β carotenone $C_{40}H_{54}O$ β apo 2 carotenol, $C_{30}H_{42}O$, β apo 2 carotenal, $C_{30}H_{40}O$ β apo 2 carotenal oxime, β-apo 4 carotenal oxime α carotene diiodide, β carotene diiodide and the products obtained by the action of phosphorus tribromide on zeaxanthin and xanthophyll

Most of these substances have one of their β ionone rings either left unaltered or modified only by the attachment of oxygen to form an epoxide Thus in dihydroxy β carotene both the hydroxyl groups are considered to be attached to the same ionone ring It will be recalled that epoxides may either revert to the original carotenoid or assume the furanoid grouping Perhaps it is significant, therefore that aurochrome, which resembles luteochrome except for having two furanoid oxygen atoms instead of one epoxide and one furanoid is not included in Karrer's list of active substances

Retinaldehyde, and other physiologically active substances which may be regarded as derivatives of vitamin A rather than of carotene, will be discussed elsewhere (Chaps 11 and 23)

The Nutritional Importance of the Various Provitamins

A more detailed description of the distribution of the provitamins in animal, vegetable and microbiological sources will be given elsewhere (Chaps 12 and 13) Further attention will also have to be given to their chemical properties, particularly in relation to their conversion to vitamin A (see Chap 17) and to their stability in foodstuffs and under biological conditions (Chaps 30 and 34)

α- or γ-carotenes will not imply the presence of much greater amounts of the more biologically potent β-carotene

In most foodstuffs moreover, it appears that provitamins are present exclusively in the all *trans* form, and without the addition of oxygen to their molecules *Cis* isomers and coloured oxidation products of the provitamins are indeed much more common as artifacts obtained during the chemical manipulation than as components of natural products In chemical estimations of provitamins in preserved foodstuffs, however, the possibility of their presence in significant amounts must not be overlooked

The predominance of cryptoxanthin as the main dietary provitamin must be of relatively common occurrence in diets based mainly on yellow maize as the staple food Such diets are habitually consumed in various parts of the world by human populations, pigs and poultry

The Synthesis of β-Carotene

This chapter may be fittingly closed by a brief mention of Karrer's crowning accomplishment in achieving the total synthesis of β carotene [11] The side chain of β ionone was extended by treatment with 3-bromopropyne to make an acetylenic carbinol Two molecules of a magnesium bromide derivative of the C_{16} compound were made to react with one molecule of 4-octene-2,7-dione to produce a tetraol This C_{40} hydroxyl carotenoid derivative was first hydrogenated with hydrogen and palladium, and then dehydrated with toluene sulphonic acid to yield β carotene Numerous other total syntheses in the carotenoid series were also effected, but space precludes their discussion in this book

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CHAPTER 9

The Isolation, Characteristics and Chemical Nature of Vitamin A

In contrast to the tendency of carotene to crystallise easily even from very impure concentrates, vitamin A will only crystallise with difficulty after it has been isolated in an almost pure state For this reason the physical characteristics of the vitamin, such as its melting point and the exact extinction coefficient for absorption at 328 mμ, remained unknown for about nine years after Karrer, Morf and Schopp [1] has obtained the vitamin in almost pure form, and had elucidated its chemical structure, except for some early uncertainty as to whether its molecule contained 20 or 22 carbon atoms

During this period progress was made in perfecting methods for the estimation of the vitamin by biological tests depending on the growth of rats, by colour tests with antimony trichloride and other reagents and by measurements of absorption in the ultraviolet region The crystallisation of the vitamin, when it was eventually acchieved did relatively little to increase our knowledge of its chemistry and biochemistry except for leading on to the problem of *cis-trans* isomerism It became possible, however, to express the results of vitamin A estimations in mg and μg rather than in International units based on spectrophotometric measurements

THE PURIFICATION OF VITAMIN A

Crystalline derivatives In 1935 the Japanese worker Kawakimi [2] obtained crystalline compounds by treating "acetylbiosterin" derived from the liver oil of the fish *Theragra chalcogramma* with maleic anhydride Two isomers were reported One formed colourless rhombic plates, melted at 261–262° C, and was sparingly soluble in chloroform The other formed retangular plates melted at 221–222° C and was easily soluble in chloroform Benzoylbiosterin was made to give crystalline derivatives in the same way

Hamano, from the same laboratory, published a separate report [3] of the same, or similar, experiments He next proceeded, however, to prepare another crystalline derivative by a slightly different method from the liver oil of *Steleopsis ishinagi* [4]. Without saponification the oil was dissolved in benzene and treated direct with maleic anhydride The crystalline deriva-

References p 96

tive so obtained melted at 220° C, and was shown to be the palmitylvitamin A dimaleic anhydride adduct derived from vitamin A palmitate naturally present in the oil

Crystalline esters of vitamin A, as distinct from adducts with maleic anhydride, were described in a third paper by Hamano [5]. By treating the unsaponifiable matter from the liver oil of *Theraga chalcogramma*, dissolved in dry pyridine, with β-naphthoyl chloride the corresponding ester of the vitamin was readily obtained in the form of yellow spindle shaped or rhombic crystals, m p 76° C Both this ester and free vitamin A which had been regenerated from it retained the biological activity of the vitamin in full A crystalline anthraquinone β-carboxylic ester, m p 124° C, was obtained

Low melting crystals of vitamin A

With the discovery of increasingly rich sources of the vitamin in various fish liver oils highly potent concentrates became more readily available and attempts to induce the vitamin to crystallise were not held up by lack of material In America Holmes and Corbet [6] dissolved concentrates prepared from the liver oils of *Stereolepsis ishinagi*, of the Atlantic mackerel and of another unnamed fish, in methanol containing a little water, and kept the solutions cold, by means of contact with solid carbon dioxide, for several days After the removal of unwanted precipitates the vitamin was obtained irrespective of the liver oil used, as pale yellow needles, m p 7 5–8° C

Spectrophotometric measurements at 328 mμ gave $E^{1\%}_{1\,cm}$ = 2100 in alcohol which is difficult to reconcile with recent readings of about 1750 for the pure substance [7] The biological activity was about 3,000 000 i u per g, which agrees with subsequent experience The molecular weight, as determined by depression of the freezing point of cyclohexane was 294, which was only 3 % higher than the theoretical value demanded by Karrer's C_{20} formula Since the crystals had to be kept cold by a vacuum jar, or by some other means when they were inspected or used in the laboratory, they were unsuitable for use as a standard for the estimation of the vitamin

Distillation methods

The early attempts by Drummond and others to purify vitamin A by the fractional distillation of its concentrates under low pressure and from an ordinary distillation flask, usually resulted in considerable destruction of the vitamin From about 1930 onwards, however attempts were made, both in England and America, to apply the relatively new technique of molecular distillation

In the old-fashioned form of distillation a thick layer of the liquid is kept boiling Bubbles of vapour rise through the liquid, and eventually reach a distant condenser This procedure allows the less volatile substances to be reliquified during the long passage towards the condenser and returned to the distillation flask, which aids the efficiency of the separation Unstable

substances, however, are damaged when they are held at a high temperature awaiting evaporation, or are evaporated more than once

In molecular distillation, in contrast, conditions are arranged to avoid these disadvantages. (1) by reducing the pressure as low as possible the mean path which the molecules will travel before they are impeded by striking another molecule is made as long as possible, (2) by making the distance between the heated liquid and the condensing surface as short as practicable collisions between molecules during distillation are minimised, (3) by spreading the liquid over a large surface for evaporation it is possible both to distil it below its boiling point at the particular pressure and to avoid keeping it at a high temperature for a long time before it can reach the surface The description 'molecular distillation" presumably implies that each molecule takes its own path from the evaporating surface to the condenser, without colliding with other molecules or forming droplets

Hickman, who became interested in high vacuum technique when working for Eastman Kodak Company on problems connected with the drying of cinema film, must be given the main credit for perfecting laboratory apparatus and works plant for molecular distillation As early as 1930 he recognised the promise of distillation as a means for concentrating the vitamins of cod liver oil, and a patent was registered in 1933 (U S Patent 1,925,559 (1933) Appl Dec 23, 1930) He soon realised that the most simple form of molecular still, consisting of a flat vessel to hold the distilland with a condensing surface placed close to it for catching the distillate, was open to serious objections Since distillation occurred without boiling there was nothing to stir up the distilland and so keep the process of distillation going by continuously exposing fresh portions of the fluid Obviously this disadvantage could be minimised by having the distilland in a very thin layer, but this remedy would mean that only small amounts of oil could be handled at each operation The oils to be distilled, moreover always contained gas, which caused uncontrolled frothing unless it was removed before distillation The preliminary heating of the oil in bulk for the removal of gas was no less objectionable than the heating entailed in the old method of distillation from a flask

Hickman therefore evolved the "cyclic" form of still[8] An evacuated system is arranged on the principles shown in Fig 6 A large cold reservoir holds a supply of the oil to be distilled, which is fed, either by gravity or by a pump, on to the heating surface The more volatile constituents of the oil evaporate and are collected on the adjacent condenser, and the rest of the oil is cooled and is collected in a second reservoir In operation the first run of the oil between the two reservoirs is made at a relatively low temperature for the purpose of removing gas from the oil The oil is then put back into the

References p 96

first reservoir, or the positions of the reservoirs are interchanged, and successive runs are made with graded increases in the temperature of the heating surface Fractions of distillate are collected during each run

When cod liver oil was submitted to cyclic distillation it first gave fractions containing free fatty acids, and the substances responsible for its fishy smell Less volatile fractions followed which contained among other constituents vitamin A alcohol, vitamin D, vitamin A esters, and a bland oil

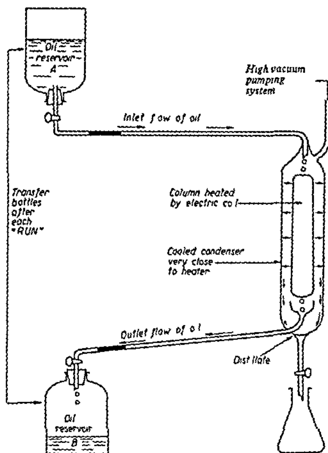


Fig 6 Diagram to explain the principle on which the molecular still is based Practical details are not shown

free from both odour and vitamins The success of the method was so complete as eventually to justify the formation of a special company, Distillation Products Industries, for its exploitation Recently the large stills used by this concern have been of the centrifugal type, with a rotating circular heating element which ensures that the oil is evenly distributed (see Plate 1)

Molecular distillates In 1932 Heilbron *et al* [9] described the successful preparation of highly potent vitamin A concentrates by distillation The distillation was carried out by Carr and Jewell of British Drug Houses by means of a molecular still with a long evaporating surface which could be heated along its length to graded temperature ranges Later

Carr and Jewell [10] described their own work with the same apparatu claimed to have produced the most potent vitamin A concentrates available with E at 328 mμ = 1600 as compared with 1370 found by bron *et al* The concentrates so obtained were pale yellow viscid oils could not be crystallised Five years of further effort at British Drug H left the vitamin still to be isolated in complete purity Mead Underhi Coward [11] however succeeded with a cyclic still designed by Hickn

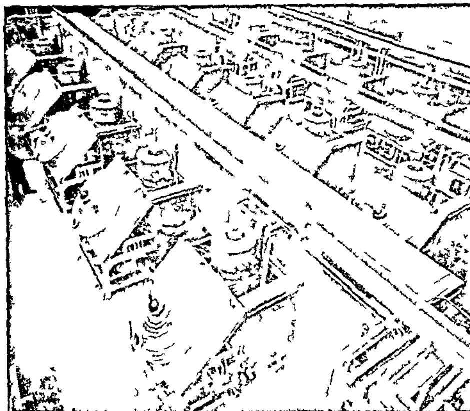


Plate 1 A group of centrifugal molecular st lls as used for the concentrat on of v A (by courtesy of Distillation Products Industries Rochester N Y U S A

confirming the claim of Holmes and Corbet that the vitamin could b tained in the form of low melting crystals They considered however these were not free from solvent The Japanese work on crystalline de tives of the vitamin in the form of its 2 naphthoate and anthraquino carboxylate was also confirmed

Success in isolating pure vitamin A however was achieved not by distillation as the final process of concentration but by its applicati untreated fish liver oils before saponification Thus Baxter and Robes of Distillation Products Industries first distilled fish liver oils in a cyclic

References p 96

which gave concentrates with E at 328 mμ = 400 or more indicating about 25 % of vitamin A These concentrates were next saponified, to give products with E = 1100–1300 Crystallisation was then found to occur easily, with or without redistillation, when 10 % solutions of the unsaponifiable matter in either ethyl formate or propylene oxide were freed from sterols and cooled to −35° C

High melting crystals of pure vitamin A and vitamin A palmitate

After they had been dried in a vacuum at a low temperature the crystals obtained by Baxter and Robeson were pale yellow prisms, free from solvent and with m p 62–63° C (Plate 2) The average extinction coefficient at 328 mμ for 18 preparations which had been crystallised twice was 1725 It was suggested that the slightly higher values which had been recorded by previous workers were due to slight differences in spectrophotometric technique In the antimony trichloride reaction E at 622 mμ was 4700 Preliminary biological tests indicated about 2,700 000 i u per g

Vitamin A palmitate was also obtained in crystalline form by esterifying crystalline vitamin A alcohol with palmityl chloride in the presence of quinoline at −15° C The crude ester crystallised readily from a 2 % solution in propylene oxide at −38° C in the form of yellow plates m p 26–28° C For specimens crystallised twice the average for E at 328 mμ was 940 which corresponds to 1720 for vitamin A alcohol

Physical and Chemical Characteristics

General properties

Vitamin A_1 $C_{20}H_{30}O$ m p 62–63° C, has $E^{1\%}_{1\,cm}$ at 328 mμ = 1750, based on the average of recent estimations by numerous workers[7] The absorption curve is not quite symmetrical, and has an inflection at about 305 mμ The vitamin is soluble in fats and in all the usual organic solvents It is insoluble in water, but may be dispersed in the aqueous phase either by emulsification or by attachment to proteins As the free alcohol it is hypophasic in the "phase test , being distributed in 80 % methyl alcohol in preference to light petroleum In contrast its esters are epiphasic It exerts no optical rotation When exposed to ultraviolet irradiation it exhibits strong yellow fluorescence, while undergoing destruction

Colour tests

When treated with dehydrating agents, such as sulphuric acid and phosphorus pentoxide, the vitamin passes through purple or blue coloured phases while undergoing rapid inactivation Rather more lasting bright blue colours may be obtained by treatment with arsenic trichloride reagent The production of colour by these reagents is held to be due to rearrangements in the positions of the unsaturated linkages (see

Plate 2 *Above* Crystalline vitamin A alcohol Magnification 8 ×
Below Crystalline vitamin A *p* phenylazobenzoate a typical artificial ester Magnification 15 × (Both by courtesy of Distillation Products Industries)

Chap 11) The vitamin also produces bright blue products when absorbed on fullers' earth which has been treated with acid and possibly on the same material activated in other ways

Instability towards oxidation Sensitivity to oxygen is one of the vitamin's main characteristics Ozonisation ultimately produces geronic acid, which gave Karrer[13] evidence of the presence of the β-ionone ring, which he had already found in carotene The vitamin is rapidly destroyed, moreover, with loss of its absorption at 328 mμ and its power to produce a blue colour with antimony trichloride, when it is exposed to atmospheric oxygen The destruction occurs when the vitamin is in pure form, when it is dissolved in organic solvents or fats, and particularly when it is mixed with powdery solids The rate of oxidation, however, is not equally rapid in all media Thus in synthetic glycerides which contain no antioxidants destruction may be rapid, but in natural fats containing antioxidants such as hydroquinone or the tocopherols, it may remain intact for long periods

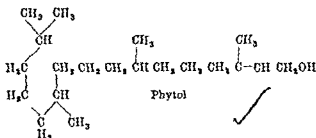
Other properties Concentrated hydrochloric acid tends to destroy the vitamin with the production of a highly characteristic absorption spectra (see Chap 11) The vitamin is very resistant to treatment with alkalis, however at least in the absence of oxygen Halogens are readily absorbed

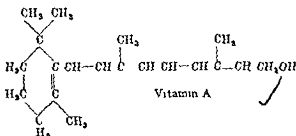
A rather remarkable feature of the vitamin's chemical behaviour is its stability in liver, blood plasma and other animal tissues during storage Provided that it is kept moist, liver may preserve most of its vitamin A even [illegible] and

Other Biological Substances with Related Chemical Structures

We may conclude this chapter with a brief account of other substances, besides congeners and derivatives (see Chap 11), in which the whole or part of the molecule has a structural resemblance to vitamin A As Karrer[13] and others have pointed out the typical carotenoid C_{40} skeleton may be derived, at least in theory, by the joining together of two molecules of phytol, a C_{20} alcohol which is of fundamental biological importance as a component of chlorophyll In the same way vitamin A may be considered as being derived from a single molecule of phytol by desaturation and ring closure In Formula Group 8 phytol has been drawn in a twisted position which shows how it might give rise to vitamin A without changing the positions of its carbon atoms

Phytol itself might be derived by the condensation of four molecules of *iso*pentane with the introduction of one double bond and a hydroxyl The tocopherols and vitamin K_1 contain phytol combined into their molecules as a side chain





Formula Group 8 The phytol molecule has been drawn in a twisted position so as to show the resemblance of its basic skeleton to that of vitamin A and the carotenoids Phytol occurs in combined form in chlorophyll vitamin E and vitamin K_1

Mention must also be made of structures which may be considered as being derived from 3 rather than 4 *iso*pentane units The alcohol farnesol $C_{15}H_{25}OH$ contains three double bonds Two farnesol molecules condensed together by conversion to farnesyl bromide and treatment with activated magnesium give squalene $C_{30}H_{50}$ [14] This hydrocarbon is present in large quantities in the livers of many sharks and of certain other animals and is interesting as an example of synthesis or metabolism proceeding towards the combination together of 6 *iso*pentane skeletons in contrast with 8 in the carotenoids and 4 in vitamin A A large number of other C_{30} substances grouped under the description triterpenes have been found in sources as widely varied as wool fat yeast and the bark of trees Two farnesol molecules joined by the hydroxyl group of one condensing with hydrogen from the terminal methyl group in the other make up the side chain of vitamin K_2

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CHAPTER 10

The Synthesis of Vitamin A

The synthesis of vitamin A proved a problem to test to the utmost the ingenuity and skill of organic chemists Although syntheses of the vitamin were claimed fairly soon after Karrer had elucidated the structural formula in 1932 it was not until 1946 that an efficient and reproducible procedure was evolved During the intervening fourteen years intensive investigations were made by many large teams of investigators Speaking in 1948 Sir Ian Heilbron [1] whose pioneer work in the field paved the way to eventual success gave expression to thoughts which must in similar circumstances have occurred to many others And so after many years he commented victory has come and the romance of exploration of high hopes and bitter disappointment will in a few years simply be recorded in text books of organic chemistry in a few terse sentences

In a book specially devoted to vitamin A a more expanded account of the steps which led to the synthesis of the vitamin is obviously appropriate Heilbron s words however will still be justified since it will be impossible if only for want of space to give a detailed history of every success and failure in the advance towards the final goal Such an account moreover would be intelligible only to those readers who have specialised in advanced organic chemistry An abridged report will therefore be given with the intention that it should be understandable to readers who are not already specialists in the field

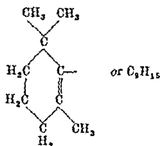
NOMENCLATURE

The first stumbling block in discussing the synthesis of vitamin A is the difficulty in giving simple names to the numerous intermediate products The starting point for most of the several syntheses which have now been achieved is the ketone β ionone $C_{13}H_{20}O$ (No I Formula Group 9) Between this compound and the final product it is sometimes practicable to describe intermediates as ionylidene derivatives *e g* β ionylideneacetaldehyde $C_{15}H_{22}O$ (II) When the application of this scheme becomes too difficult

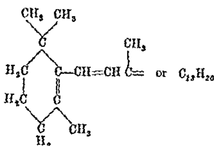
References p 112

however, the higher intermediates must be described by their complicated systematic names, by the number of their carbon atoms and their general chemical nature, or by Roman numbers referring to a series of structural formulae drawn out in full at some other place in the text.

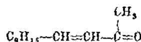
The chemical name for vitamin A may be given as 1-hydroxy-3,7-dimethyl-9-[2', 6', 6'-trimethylcyclohexenyl]-nonatetra-2, 4, 6, 8-ene. The way in which this name is derived is shown in Formula Group 10



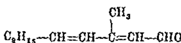
The 2, 6, 6 trimethylcyclohexenyl grouping



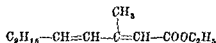
The β ionylidene grouping



(I) β Ionone, $C_{13}H_{20}O$



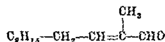
(II) β Ionylideneacetaldehyde, $C_{15}H_{22}O$



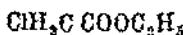
(III) Ethyl β ionylideneacetate



(IV) The glycide derivative of (III)



(V) Heilbron's C_{14} aldehyde



(VI) Ethyl monochloracetate

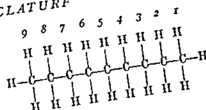
Formula Group 9 Some of the key groupings and intermediate compounds studied during early attempts to synthesise vitamin A

Reactions Used in Polyene Syntheses

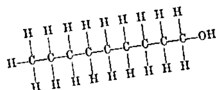
Discussion of the synthesis of vitamin A will also be complicated by references to certain well-known chemical procedures Some of these have general importance throughout organic chemistry, but others find their main application in polyene chemistry. A brief list may therefore be useful

Condensations In building up the long side chain of vitamin A it is necessary to join shorter chains together For this purpose the Grignard reagents are made by treatment of the required alkyl halide with magnesium They may conveniently be obtained from acetylenic hydro

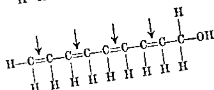
Number of carbon atom



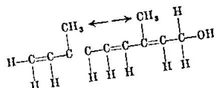
Nonane



1 Hydroxynonane

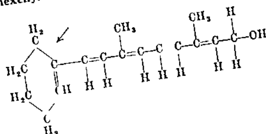


1 Hydroxynona-**2, 4, 6, 8 tetraene**

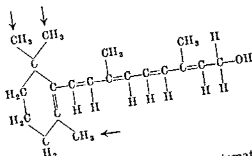


1 Hydroxy **3 7 dimethyl**nona 2 4, 6 8 tetraene

1 Hydroxy 3 7 dimethyl 9 **cyclohexenyl**nona 2 4 6 8 tetraene



1 Hydroxy 3 7 dimethyl 9 [2, 6 6 **trimethyl**cyclohexenyl] nona 2 4, 6 8 tetraene = Vitamin A $C_{20}H_{30}O$



Formula Group 10 Demonstration of the method by which the systematic name of vitamin A was derived

References p 112

carbons, however, by allowing the hydrocarbon to replace ethane from ethyl magnesium bromide Presumably this transference depends on the presence of an active or acidic hydrogen in the acetylenic grouping The Grignard compounds so obtained may be joined on to ketones or aldehydes

Reformatsky's reaction has also been successfully applied The condensation in this case is made, under the action of zinc, between a halogen substituted ester and a ketone

Ketones may also be combined with acetylenic hydrocarbons by the action of metallic sodium in liquid ammonia

Reductions Controlled catalytic hydrogenation, with a suitably chosen catalyst, may be used to reduce triple bonds in the carbon chain to double bonds.

Reduction of aldehyde groups to the corresponding alcohol may be effected by the Ponndorf reduction, which works in the reverse direction to the Oppenauer oxidation The aldehyde is treated with aluminium *iso* propoxide in the presence of a hydrogen donor. Alternatively the reduction may be induced by lithium aluminium hydride

Dehydrations Various dehydrating agents may be used for the removal of hydroxyl groups, including anhydrous oxalic acid and substituted sulphuric acids such as toluene-*p* sulphonic acid It is important to remember that double bonds are introduced at the sites of dehydration

Rearrangements After two unsaturated compounds have been condensed together it is often found possible to change the positions of the double bonds and the hydroxyl groups by treatment with iodine or dilute acids It seems remarkable to the non-specialist that such treatment so often tends to produce effects in the direction required for the synthesis of vitamin A

β-Ionone and its Reactions

According to Karrer [5] β-ionone may be obtained from the balsam of *Boronia megastigma* It may also be synthesised from the aldehyde, citral, which is readily obtained from many essential oils, including oil of verbena, oil of lemon grass and lemon oil Citral, $C_{10}H_{16}O$, is condensed with acetone in the presence of weak alkalis to give *pseudo* ionone, which is rearranged into a mixture of α- and β ionones on treatment with dilute sulphuric acid The α form may be separated from the β form by the formation of its bisulphite compound, which readily crystallises from aqueous solution

The synthesis of vitamin A from β-ionone [1] obviously requires the formation of a longer side chain to the trimethylcyclohexenyl ring For this step the condensation of an unsaturated aliphatic compound with the oxygen of the ketone group is required and the familiar Grignard reaction seems

appropriate It was found however that whereas α ionone gave a normal Grignard reaction β ionone reacted abnormally Thus Karrer *et al* [3] had no difficulty in condensing α ionone with allyl bromide in the required manner but obtained unexpected products when the same reaction was attempted with β ionone Presumably the Grignard compound reacted at some point in the unsaturated system of β ionone rather than at the carbon atom required

Karrer and his colleagues however found that β ionone combined normally with ethyl bromacetate according to the Reformatsky reaction to give ethyl β ionylideneacetate (III) This compound could readily be hydrolysed and reduced by the Bouveault Blanc method with sodium and ethyl alcohol to a saturated alcohol $C_{15}H_{29}OH$ Further condensations and other reactions with diethyl malonate and zinc methyl iodide eventually gave perhydrovitamin A $C_{20}H_{39}OH$ [4] The product was quite devoid of biological activity but was theoretically important since it proved to be identical with perhydrovitamin A prepared by the catalytic reduction of the natural vitamin Final proof was therefore given of the correctness of Karrer s structural formula

Ruzicka and Fischer [5] followed with a synthesis of tetrahydrovitamin A The double bond of β ionone was catalytically hydrogenated and the dihydro β ionone so obtained was first condensed with acetylene in presence of sodamide and then submitted to various treatments which included a further condensation with ethyl sodio acetoacetate In spite of the presence of the β ionone ring the final product had no biological activity

Early Syntheses of Impure Vitamin A and its Ethers

The first synthesis of material having growth promoting activity was reported in 1937 by Kuhn and Morris [6] β Ionone was first condensed with ethyl bromacetate to give ethyl β ionylideneacetate (III) This ester was next converted to β ionylideneacetaldehyde (II) by a complicated series of steps These included the conversion of the ester to its *o* toluidide treatment with phosphorus pentachloride to form the corresponding chloro imide reduction and removal of the chlorine with chromous chloride to yield the *o* tolyl compound of β ionylideneacetaldehyde and finally removal of the toluidine by treatment with oxalic acid to leave the free aldehyde The aldehyde was then condensed with 3 methylcrotonaldehyde $(CH_3)_2C$ CH CHO in the presence of piperidine acetate to form a C_{20} aldehyde which could be reduced to vitamin A by the Ponndorf reaction with aluminium *iso*propoxide

Unfortunately Kuhn s procedure resulted in formation of many substances besides vitamin A Even after partial purification of the final product by

References p 112

adsorption on alumina it only contained about 5% of vitamin A as measured by biological tests. Attempts by several investigators to repeat the synthesis, moreover, were unsuccessful [7-11].

Eventually it was found possible, however, to synthesise Kuhn's C_{18} aldehyde by other means, which suggested that its production by the original procedure was difficult rather than impossible [12, 13, 14].

Another early claim to have synthesised a biologically active derivative of vitamin A was made by Kipping and Wild [15]. β-Ionone was condensed in the presence of lithium with the ether $BrCH_2 \cdot CH : CH \cdot C(CH_3) : CH \cdot CH_2OCH_3$ to give a product which could be dehydrated to vitamin A methyl ether. This claim, however, has not been substantiated.

Synthesis of a C_{14} Aldehyde

The great difficulty experienced in obtaining reasonably good yields of β-ionylideneacetaldehyde, $C_{15}H_{22}O$, induced workers to look for some more readily available intermediate as a stepping stone between the C_{13} molecule of β-ionone and the C_{20} molecule of vitamin A. A valuable contribution in this direction was made in 1937 by Ishikawa and Matsuura [16] who condensed β-ionone with ethyl chloracetate. Although this procedure would appear to differ little from that of Kuhn and Morris, who had used ethyl bromacetate, conditions were so arranged as to cause the formation of a glycide ester (Formula Group 9, IV). On treatment with alkali this ester lost both its ethyl group and carbon dioxide, and gave a poor yield of a C_{14} aldehyde, which was presumed to have retained the original structure of β-ionone as far as the C_{13} atom.

Heilbron, Johnson, Jones and Spinks [9] repeated the Japanese work with specially purified β-ionone. They succeeded in obtaining the same C_{14} aldehyde, but demonstrated that the double bond in the side chain had migrated one position further away from the hexacyclic ring, giving the structural formula V. It was thought probable that this compound would give a normal Grignard reaction, and a scheme for the synthesis of the vitamin was suggested in which the C_{14} aldehyde was to be condensed with acetylene (C_2) and 3-ketobutanol (C_4). Unfortunately restrictions on research imposed by war prevented Heilbron and his colleagues from achieving the success which must inevitably have attended a full scale attempt to put their theories into practice.

The Synthesis of Pure Vitamin A

In 1946, about 4 years after Heilbron had indicated his procedure for its synthesis, the vitamin was at last produced in pure form by the intensive efforts of workers in commercial firms. The ingenuity, skill and patience of

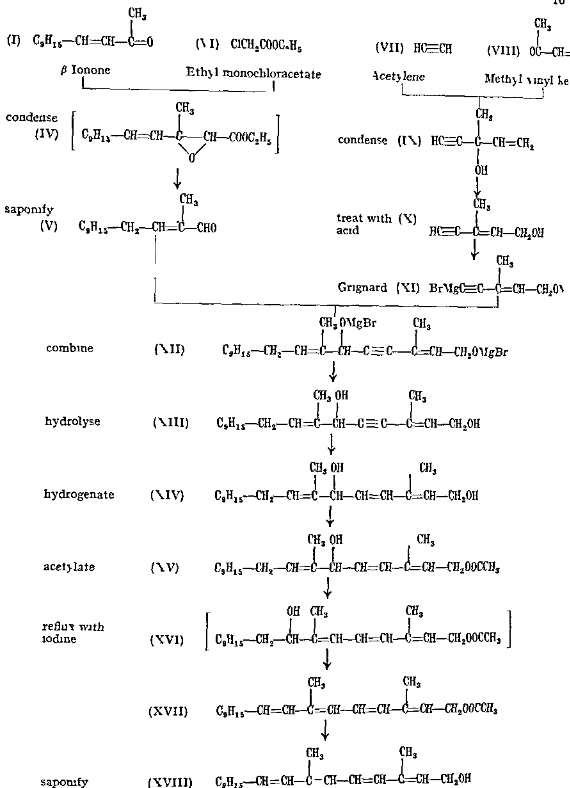
the investigators concerned must be fully admitted and praised. The final achievement, however, was probably due more than anything else to the concentration of large technical resources to attack a problem of such great commercial importance It is perhaps significant that several routes for the synthesis were eventually described almost simultaneously, and that many different procedures are now available Thus suggests that the synthesis of vitamin A has been achieved not by the solution of a single narrow problem, but by the application of wide knowledge which has been gained by long and patient research on polyene chemistry

For their parts in the final synthesis honour must be given to Isler and his colleagues, to Milas, and to Arens and Van Dorp As early as 1945 Milas took out patents (Nos 2,369 156–2,369,168, 2,382,085 and 2,382,086) with the U.S. Patents Office, which described processes which were later fully vindicated as being valid for the synthesis of the vitamin In 1947 Arens and Van Dorp followed, in the author s opinion, with the first publication in a scientific journal of a clear description of a successful synthesis In neither of these claims, however was adequate proof given of the production of large quantities of the vitamin in pure form Credit for the description of this crowning achievement is due to Isler and his colleagues in Basle.

Isler, Huber, Ronco and Kofler [17] gave a fully documented account of their procedure in 1947, and it may be summarised here as a typical synthesis based on Heilbron's suggestions The four starting materials were β-ionone, ethyl monochloracetate, acetylene and methylvinyl ketone The procedure is shown in Formula Group 11, which may be supplemented with the following notes

Left hand side of molecule (on paper) β Ionone $C_{13}H_{20}O$ (I) was condensed with ethyl monochloracetate (VI) by stirring in the cold with the addition of dry powdered sodium alcoholate. Treatment with methanolic sodium hydroxide followed The glycide acid ester (IV) which was first formed but not isolated was saponified and decarboxylated to give the aldehyde $C_{14}H_{22}O$ (V) which had been described by Heilbron *et al* [9] After purification the yield corresponded to 80% of the original β-ionone

Right hand side of molecule Acetylene, C_2H_2 (VII) was condensed with methyl vinyl ketone, C_4H_6O (VIII) under the action of sodium in liquid ammonia to give the tertiary alcohol 3 hydroxy-3-methylpentenyne (IX) Treatment of this compound with dilute sulphuric acid caused the hydroxyl group to migrate to the end of the molecule remote from the triple bond, and the double bond to migrate towards the triple bond, with the production of the unsaturated primary alcohol 1 hydroxy-3-methylpent-2-en-4-yne or C_6H_8O (X)



Formula Group 11 Synthesis of pure vitamin A by Isler *et al* The intermediate compounds shown in brackets were not isolated

Combination of right and left sides. Grignard reagent was first made by treating magnesium shavings in the cold with ethyl bromide. The alcohol C_6H_8O (X), which had already been prepared was then added, and the mixture heated, to form the corresponding dimagnesium bromide salt of the alcohol (XI). The grey mass so obtained was cooled, and the C_{14} aldehyde (V) was added in ether, which was refluxed for 2½ hours so as to form the Grignard product (XII). Hydrolysis with ammonium chloride and ice followed, and the C_{20} dihydroxy compound (XIII) was purified in various ways, including the removal of the excess of C_6H_8O alcohol by distillation, solution in light petroleum, and transfer to 75% methyl alcohol. The yield was 81% in relation to the amount of C_{14} aldehyde.

Partial hydrogenation, dehydration and rearrangement of the resulting C_{20} alcohol It will be noticed that at this stage a C_{20} molecule (XIII) had been obtained, which resembled vitamin A in having a correct β-ionone ring, a terminal hydroxyl group at the end of the carbon side chain and the two methyl groups in the side chain in their correct 3,7 positions On the other hand an unwanted hydroxyl was present at C_6, and the arrangement of the unsaturated linkages between C_4 and C_8 was incorrect Thus instead of double bonds at C_4, C_6 and C_8 there was a triple bond at C_4 and a double bond at C_7

The final steps of the syntheses started with the reduction, by hydrogen and a palladium lead catalyst suggested by Lindlar, of the triple bond, to give a 97% yield of the dihydric alcohol (XIV) Partial acetylation with acetyl chloride followed (XV), in order to protect the terminal hydroxyl group It now only remained to reflux the acetate with iodine in light petroleum of b p 80–110 °C to give vitamin A acetate. This procedure

TABLE 7

COMPARISONS BY ISLER *et al* [17] BETWEEN SPECIMENS OF VITAMIN A OF SYNTHETIC AND NATURAL ORIGIN

Vitamin A		*m p*	*Mixed m p*	$E^{1\%}_{1cm}$ *328mμ (max)*	$E^{1\%}_{1cm}$ *260mμ (min)*
Alcohol	Synth	60–62°	60–63°	1720	180
	Nat	62–64°		1680	180
Anthraquinone β-carboxylate	Synth	122–123°	122–123°	1080	
	Nat	122–123°		1120	
β-Naphthoate	Synth	74–75°	74–75°	1210	220
	Nat	74–75°		1210	220
p-Phenylazobenzoate	Synth	79–80°	79–80°	1650	200
	Nat	79–80°		1550	200

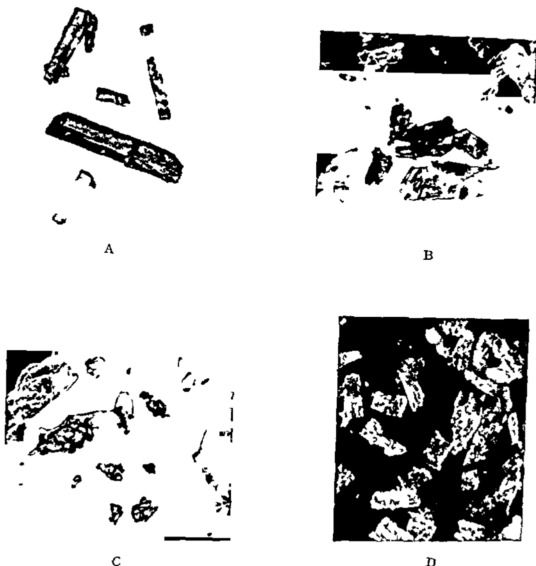
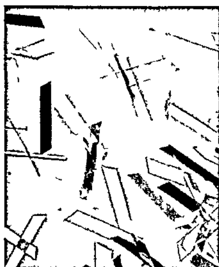


Plate 3 Microphotographs of vitamin A and some of its esters Low magnification (by courtesy of H Waldmann of F Hoffmann La Roche & Co, Ltd)

A Synthetic vitamin A alcohol, m p 60–62°
B Anthraquinone-β carboxylate, m p 122–123°
C β Naphthoate, m p 74–75°
D p-Phenylazobenzoate, m p 79–80°



A



B



C

Plate 4 Recent microphotographs of vitamin A esters High magnification (by courtesy of H Waldmann of F Hoffmann La Roche & Co Ltd)

A Anthraquinone β carboxylate m p 122 123°
B β Naphthoate m p 74–75°
C p Phenylazobenzoate m p 79 80°

References p 112

first caused an allyl migration of the double bond from C_7 to C_6 and a corresponding shift of the hydroxyl group from C_6 to C_8 (XVI) Dehydration then occurred between C_8 and C_9 to introduce the fourth double bond of the side chain into its correct position (XVII)

Free vitamin A alcohol (XVIII) was readily prepared from its ester by saponification and was shown by spectrophotometric colorimetric and biological tests to be identical with the natural product Its anthraquinone carboxylic acid naphthoic acid and p phenylazobenzoic acid esters were also identified with the same esters made from the natural vitamin (see Table 7 and Plates 3 and 4)

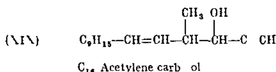
Other Routes of Synthesis

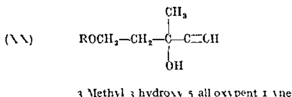
From the C_{14} aldehyde In 1946 Milas[18] also described the synthesis of esters and ethers of vitamin A by methods which were essentially similar to those suggested by Heilbron (Formula Group 12) In his first method a C_{14} aldehyde was first prepared by much the same technique as was used by Isler Since the aldehyde produced geronic acid on treatment with ozone however it was concluded that the double bond in the side chain had remained in the C_3 position it occupies in β ionone and had not changed to the C_2 position as stated by Heilbron The C_{14} aldehyde was treated with lithium acetylide to give a C_{16} compound (XIX) which was next condensed by a Grignard reaction with a ketone of the type $CH_3COCH_2CH_2OR$ This produced a C_{20} compound with two hydroxyl groups in the side chain and with the end of the chain in the form of an ester or ether according to whether R was an acyl or an alkyl group By dehydration the unwanted hydroxyls were removed from the side chain and replaced by double bonds and by partial hydrogenation the triple acetylene linkage was reduced to another double bond to give derivatives of the vitamin

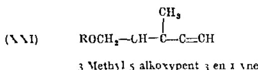
The second method of Milas resembled that of Isler in attaching the acetylene to the portion of the side chain to be added rather than to the C_{14} aldehyde The condensation to obtain the C_{20} skeleton was by the Grignard technique between the C_{14} aldehyde and 3 methyl 3 hydroxy 5 alkoxy pent 1 yne (XX) The third method differed in including the preliminary dehydration of the side chain fraction to 3 methyl 5 alkoxypent 3 en 1 yne (XXI) before the application of the Grignard condensation

It is evident that all these methods and that of Isler are essentially the same and that they differ mainly in the order in which the various steps are undertaken It seems significant however that Isler and his colleagues produced vitamin A of full biological activity in substantial yield In contrast the products of Milas at least as described in 1946 had relatively

low biological activities varying from 1 5 to 30% of that of the natural vitamin





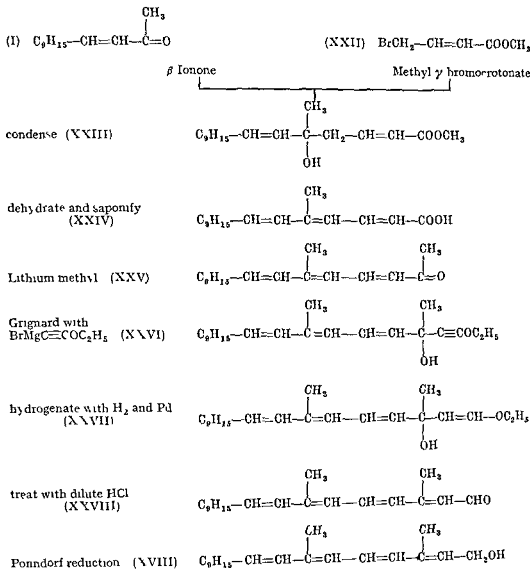


Formula Group 12 Key intermediates involved in synthesis of vitamin A and its derivatives by Milas *et al*

Other routes Van Dorp and Arens [19, 20] were responsible for a synthesis along different lines (Formula Group 13) β Ionone was first condensed with methyl-γ bromocrotonate (XXII) by zinc in benzene to give a hydroxy ester (XXIII) which was dehydrated with anhydrous oxalic acid and then saponified to give ionylidenecrotonic acid $C_{17}H_{24}O_2$ (XXIV) as the main product This acid was readily converted into the C_{18} ketone (XXV) by treatment with lithium methyl The synthesis then proceeded by the addition of ethoxyacetylene by the Grignard reaction to form the acetylenic hydroxy ether (XXVI) partial hydrogenation to form the ether (XXVII) rearrangement and hydrolysis by treatment with dilute hydrochloric acid to form vitamin A aldehyde (XXVIII) and finally reduction with lithium aluminium hydride to form vitamin A (XVIII) A synthesis by Karrer Jucker and Schick [21] was also based on the combination of β ionone with bromocrotonic acid as its first step

Finally a new procedure by Attenburrow *et al* [22] is specially interesting for the use of 2 methylcyclohexanone (XXIX) in place of β ionone as the starting material (Formula Group 14) Additional methyl groups were first introduced in two stages by treatment with methyl iodide and sodamide in a non polar solvent At each stage the desired intermediates (XXX) and (XXXI) were purified from unwanted products by separation on a column packed with gauze The trimethyl compound (XXXI) was easily converted to 1 ethynyl 2 2 6 trimethylcyclohexanol $C_{11}H_{18}O$ (XXXII) by treatment with sodium acetylide in liquid ammonia A Grignard reaction with the

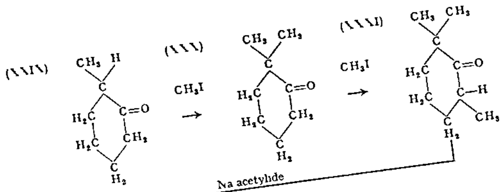
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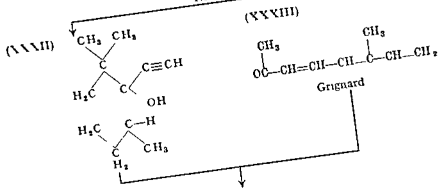


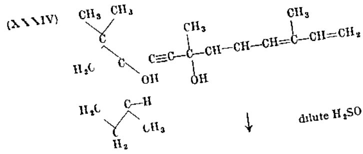
Formula Group 13 Synthesis of vitamin A by Van Dorp and Arens

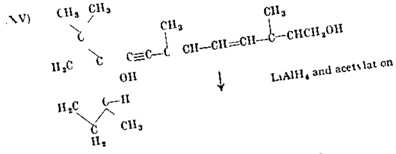
unsaturated ketone (XXXIII) $C_9H_{12}O$ gave a C_{20} glycol (XXXIV) which was rearranged by treatment with dilute sulphuric acid. One of the hydroxyl groups was thus made to migrate to the required position at the end of the side chain (XXXV). The triple bond was next reduced to a double bond by means of lithium aluminium hydride, and the product was acetylated (XXXVI) Treatment with toluene-*p*-sulphonic acid, which removed the hydroxyl from the ring and also the terminal acetyl group, gave vitamin A (XVIII)

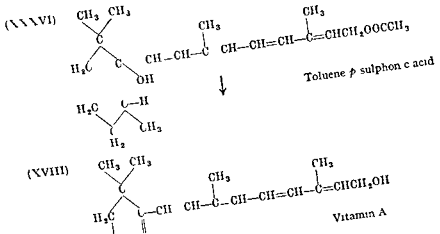
Formula Group 14 *(See p 111)* Synthesis of vitamin A from 2 methylcyclohexanone by Attenburrow *et al*


(XXIX)
(XXX)
CH$_3$I
(XXXI)
CH$_3$I


Na acetylide
(XXXII)
(XXXIII)
Grignard


(XXXIV)
dilute H$_2$SO$_4$


LiAlH$_4$ and acetylation


(XXXVI)
Toluene p sulphonic acid
(XVIII)
Vitamin A

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CHAPTER 11

The Chemistry of the Congeners and Derivatives of Vitamin A

There are many natural congeners of vitamin A and to complicate the picture further numerous artificial derivatives have been described Some of these substances are biologically active but others are inactive Some are important for practical or theoretical reasons but others seem less interesting So much detailed information is available that our account cannot be fully comprehensive It is difficult moreover to group the congeners in any very logical order based on their chemical properties After careful thought it seemed advisable to start by reviewing all the derivatives of vitamin A_1 grouped according to both their mode of formation and their absorption spectra Vitamin A_2 and its corresponding derivatives could then be dealt with more shortly at the end of the chapter

The *cis trans isomers* of vitamin A_1 are mentioned first because they differ so little in structure from the parent substance Next we take *retinene* (vitamin A aldehyde) in recognition of its natural occurrence and great physiological interest *Vitamin A acid*, which is an interesting synthetic product follows by virtue of its chemical derivation from the aldehyde and its similarity in having its absorption maximum at a longer wavelength than that of vitamin A The synthetic *vitamin A ethers* are dealt with next as further derivatives resulting from modifications at the end of the side chain of the vitamin

Our attention is next turned to *anhydrovitamin A* an artificial derivative with a highly characteristic absorption spectrum which has proved valuable in examining unknown substances believed to be related to the vitamin *Rehydrovitamin A* comes next as a substance produced from anhydrovitamin A in the animal body and *axerophthene* as a product of chemical reduction

We next proceed to *hepaxanthin* or *vitamin A epoxide* and oxidation products of the vitamin which are presumably derived by the epoxide route *Sub-vitamin A* may be brought under this heading

Kitol is next considered as a natural condensation product of vitamin A and *homovitamin A* as a synthetic product with a lengthened side chain

References p. 126

Finally we have *vitamin A_2* a more unsaturated compound than vitamin A_1 and its products

In this chapter only brief mention will be made of the biological activity of vitamin A derivatives This interesting subject will be treated more fully however in Chapter 23

Derivatives of Vitamin A_1

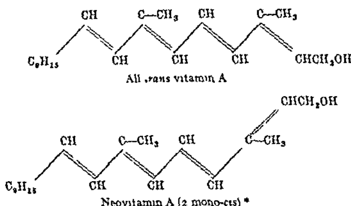
Neo (or cis) vitamin A In 1945 Robeson and Baxter[1] described the isolation of a new form of vitamin A from the mother liquor from which the usual form had been separated It crystallised as light yellow needles m p 59–60 °C in contrast to 62–64 °C for the prisms of vitamin A_1 as previously characterised $E^{1\%}_{1\,cm}$ at 328 mμ was 1675 as against 1750 at 325 mμ for the old form The anthraquinone carboxylate was red and melted at 130 131 °C whereas the same derivative of the old form was yellow and melted at 123–124 °C The new form was decidedly more stable to oxidation than the old and reacted less rapidly with maleic anhydride



Plate 5 Neovitamin A (by courtesy of Distillation Products Industries) cf all *trans* vitamin A shown in Plate 2 page 93

It was responsible for about one third of the activity of most fish liver oils and was assumed to be an isomeride of the old form with the *cis* configuration at the double bond nearest the hydroxyl group (Plate 5)

Later the name 'neovitamin A" was given in accordance with the practice for describing *cis* isomerides[2] At least four *cis* isomers of vitamin A_1 seemed possible, and it was suggested that the form isolated had a *cis* linkage at the double bond nearest to the hydroxyl group



All *trans* vitamin A

Neovitamin A (2 mono-cis) *

Retinene (Vitamin A aldehyde) Retinene has great biological importance as a component of visual purple (Chap 22) and probably as an intermediate product in the biological conversion of carotene to vitamin A (Chap 17) Morton[3] made a brilliant advance in recognising that the spectroscopic properties of Wald s retinene[4] were identical with those of vitamin A aldehyde although at the time of this discovery he thought that visual purple was derived from riboflavin rather than from vitamin A Soon afterwards Morton and Goodwin[5] prepared vitamin A aldehyde from vitamin A by oxidation with potassium permanganate and identified it both with retinene from eyes and with vitamin A aldehyde as previously obtained by Hunter and Hawkins[6] from vitamin A by oxidation with aluminium *iso*propoxide Thus the absorption maxima at 368 mμ in cyclohexane and at 664 mμ in the antimony trichloride reaction were in good agreement after correction for different solvents with values reported for material from the other two sources

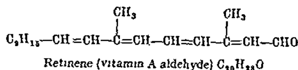
Later Ball, Goodwin and Morton[7] found that vitamin A could be readily converted to retinene by allowing a solution in light petroleum to stand in the dark for a few days in the presence of manganese dioxide By choosing the right conditions yields of up to 80% were obtained The retinene was purified by chromatography, and after recrystallisation from light petroleum at −72 ° gave clusters of large orange red crystals, predominently needle shaped, which melted at 61–62 °C $E^{1\%}_{1\,cm}$ at 373 mμ in cyclohexane was 1548, and at 664 mμ in the antimony trichloride reaction 3400 Retinene forms crystalline derivatives such as the 2,4-dinitrophenylhydrazone and the semicarbazone With amino compounds and proteins it gives yellow or

* Geneva system of numbering

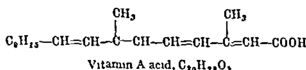
References p 126

red addition compounds which are interesting in their resemblance to the early breakdown products of visual purple [8] Retinene has high biological activity It may readily be reduced to vitamin A by chemical methods (see Chap 10)

A substance absorbing at 560 mμ in the antimony trichloride reaction was observed by Morton and Goodwin as side product in the oxidation of vitamin A to retinene Later a similar product, with absorption at 345 mμ in chloro form, was purified by Meunier and his colleagues [9, 10] and was considered to be an epoxide of retinene The biological activity was only 4% of that of vitamin A



Retinene (vitamin A aldehyde) $C_{20}H_{28}O$



Vitamin A acid, $C_{20}H_{28}O_2$

Vitamin A acid This substance has only been obtained artificially, but it is of great theoretical interest in having high biological activity without undergoing conversion to the vitamin itself (see Chap 23) Its ethyl ester was apparently synthesised by Heilbron *et al* [11], but the acid itself was not purified or tested biologically Van Dorp and Arens[12] later reported the synthesis of the acid in pure form β Ionone and γ-bromocrotonic methyl ester were combined together, and dehydrated and saponified to give β-ionylidenecrotonic acid This product was then trans formed by means of lithium methyl into a C_{18} ketone which was converted to vitamin A acid by treatment with methyl bromacetate followed by dehydration and saponification

The acid forms pale yellow crystals m p 181 5 °C In ethyl alcohol it has an absorption band at 347 mμ, with $E^{1\%}_{1\,cm}$ 1460 With antimony trichloride a red colour with a bluish lustre has been reported but the colour is presumably much less intense than that produced by vitamin A

Vitamin A ethers Vitamin A methyl ether is another artificial product which has strong biological activity Its synthesis by Isler and his colleagues [13] preceded the synthesis of the vitamin itself β-Ionone was first converted to the corresponding C_{14} aldehyde, which was then combined with 1-methoxy-3 methylpent 2 en-4 yne The methyl ether was also prepared as a derivative of natural vitamin A by Hanze *et al* [14] It gave light yellow crystals m p 33–34 °C after prolonged storage of a solution in methyl alcohol at –70 °C With allowance for the difference in molecular

weight the ether had virtually the same absorption spectrum as vitamin A, $E^{1\%}_{1\,cm}$ at 326 mμ being 1660. Later Isler and his colleagues [15] synthesised butyl and phenyl ethers, which were found to have much lower biological potency than the methyl ether.

Anhydrovitamin A. It is still doubtful whether this substance occurs otherwise than as an artifact obtained during the chemical manipulation of vitamin A. Its discovery originated in attempts by Edisbury *et al* [16] to study the changes in vitamin A which occurred when a blue colour was produced with antimony trichloride. The vitamin, in the form of cod-liver oil, or various concentrates, was treated with the reagent for one minute. Water was then added, and the chloroform layer separated by dissolving the precipitated antimony oxychloride with hydrochloric acid Spectroscopic examination of the recovered material, in chloroform solution, indicated that the broad band of vitamin A at 328 mμ had been replaced by a series of narrow bands at 280, 324, 340, 357, 376, 399 and 425 mμ

Similar changes could be produced by treating an alcoholic solution of the vitamin with dry hydrogen chloride, which caused the appearance of well defined maxima at 333, 350, 369 and 392 mμ, as observed in alcohol (Fig 7).

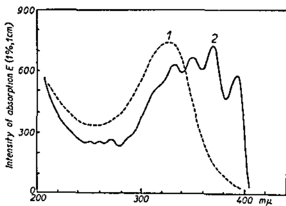


Fig 7 Formation of anhydrovitamin A and related products The broken line shows the absorption spectrum of a concentrate rich in vitamin A The continuous curve shows the spectrum of the same concentrate after treatment with dry hydrogen chloride (after Edisbury *et al*)

The same type of spectrum also appeared occasionally when rich concentrates of the vitamin were submitted to slow vacuum distillation [17]. Thus a distillate from a halibut-liver concentrate had maxima at 297, 313 5, 330, 348, 368 and 393 mμ At the time the change from the single broad band of vitamin A to the fine structured spectrum was attributed to cyclisation, with the formation of hydronaphthalene derivatives Heilbron, Morton and Webster [18] demonstrated that 1,6-dimethylnaphthalene could certainly be obtained by refluxing a vitamin A concentrate with selenium. Its absorption

References p 126

maxima, however, were far removed from those seen after the treatment with hydrochloric acid

The occurrence of "cyclised" or "spurious" vitamin A in the more volatile distillates from tuna and other fish-liver oils was later reported by Embree[19] A distillate was obtained which had maxima at 350, 368 and 389 mμ with $E^{1\%}_{1\,cm}$ at 368 mμ = 1385 The shape of the curve was identical with that produced by the action of hydrogen chloride on vitamin A The formation of the substance could be promoted by heat, but the extent of heating during distillation was considered to be insufficient to account for the amounts found in the distillates It therefore appeared to be present in the oils naturally, but nevertheless to be biologically inactive Embree pointed out that the substance with the same absorption spectrum had previously been separated by Castle, Gillam, Heilbron and Thompson[20] as the least strongly adsorbed fraction obtained from vitamin A concentrates by chromatography

Some years later doubts as to the correctness of the description "cyclised" vitamin A were raised by Meunier, Dulou and Vinet[21] Thus the spectroscopic changes could be produced in vitamin A by treatment with phosphorus bromide followed by potassium iodide, a procedure known to cause dehydration It was concluded that the loss of water caused the introduction of a new double bond into the molecule, and that the previous five double bonds were shifted to the left, as the molecule is usually drawn The dehydration product was yellow by itself, but blue when ionised under the influence of antimony trichloride, or on adsorption on acid china clay The name "axerophthene" was proposed, but is better reserved for a hydrogenation product which will be described later

The same view about the absence of cyclisation was reached independently by Shantz Cawley and Embree[22] By methods which included chromatography and crystallisation from light petroleum they isolated the anhydro compound in the form of orange crystals, which melted at 76–77 °C. Three absorption bands were shown at 351, 371 and 392 mμ, with $E^{1\%}_{1\,cm}$ = 2500, 3650 and 3180 respectively (Fig 8) Presumably the previous observation of several more maxima after the treatment of vitamin A with hydrochloric acid indicates that changes other than direct conversion to a single anhydro derivative can readily occur In antimony trichloride reaction $E^{1\%}_{1\,cm}$ at 620 mμ was 5500, which is somewhat higher than for vitamin A Elementary analysis and a negative Zerewitinoff reaction for hydroxyl groups proved that the compound is a hydrocarbon produced by dehydration and not a cyclisation product The name "anhydrovitamin A' was given Very slight biological activity was detected

As a further observation Embree and his colleagues noticed that by

prolonging the action of hydrochloric acid on vitamin A another product "isoanhydrovitamin A" could be obtained. It had bands at 330, 350 and

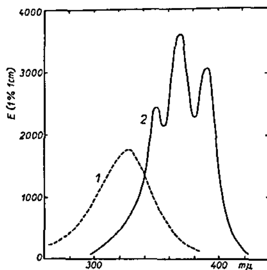
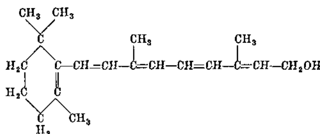
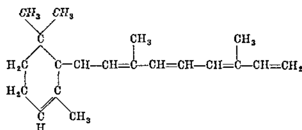


Fig. 8. The absorption spectrum of anhydrovitamin A (after Shantz *et al.*). The broken curve shows the spectrum of vitamin A and the continuous curve the spectrum of *anhydrovitamin A. Note the increased intensity of absorption.*



Vitamin A (5 double bonds)



Anhydrovitamin A (6 double bonds)

370 mμ. The use of stronger acid produced dark orange and red polymers. Meunier and his colleagues[23] confirmed the formation of *iso*anhydrovitamin A, but found that even more prolonged treatment gave "substance

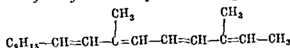
References p. 126

x", which resembled vitamin A in having a single band at 325 mμ It appeared to contain combined HCl, but insufficient to satisfy the formula $C_{20}H_{29}Cl$ Anhydro and *iso*anhydrovitamin A both had slight biological activity, but "substance x" was inactive

Both Embree and Meunier realised the importance of the spectral changes on treatment with hydrochloric acid as a guide to structure in examining substances believed to be related to vitamin A Another conclusion which can be drawn from their work is that neither an absorption band at 328 mμ nor a band at 620 mμ in the antimony trichloride reaction can be completely trusted as a guide to biological activity in products which have been subjected to certain forms of chemical treatment

Rehydrovitamin A A substance with absorption bands at 351 and 369 mμ, and with an inflection at 330 mμ, was detected by Shantz [24] in the livers of rats which had been given large doses of anhydro vitamin A Its chromatographic behaviour before and after saponification indicated that it was laid down in the liver in the form of esters, from which a free alcohol could be obtained by hydrolysis It could not be reconverted to anhydrovitamin A by dehydration In biological tests it was much more potent than anhydrovitamin A

Axerophthene This substance, which Karrer has described as the fundamental hydrocarbon corresponding to vitamin A (axerophthol), differs from the vitamin only in having a hydrogen atom in place of the hydroxyl group It was synthesised as a light yellow liquid by Karrer and Benz [25] by treating the corresponding C_{18} ketone with ethyl magnesium bromide Although its system of double bonds is presumably the same as in vitamin A, axerophthene has three maxima 331, 346 and 364 mμ with $E^{1\%}_{1\,cm}$ of 1080, 1260 and 952 respectively Two maxima at 474 and 577 mμ are seen in the antimony trichloride reaction von Euler and Karrer [26] have reported that the biological potency is about one fifth of that of vitamin A Their suggestion that axerophthene is formed *in vivo* after the administration of anhydrovitamin A will justify its description at this point in our test



Axerophthene ($C_{20}H_{30}$)

Vitamin A epoxides and related products We have seen that in the formation of retinene oxidation takes place at the end of the side chain of vitamin A, and that the absorption band is shifted towards longer wavelengths We have now to discuss vitamin A epoxide, in which oxygen is presumably attached to the molecule between two carbon atoms in the ring, at the point of attachment of the side chain Oxidation at

this point causes the absorption band to be moved towards shorter wavelengths In addition to vitamin A epoxide itself we shall also have to mention a series of related derivatives, not yet identified, whose existence must be assumed from the complex absorption spectra which appear during the oxidation of vitamin A by air or by certain oxidising agents

In 1932 Van Eekelen, Emmerie, Julius and Wolff [27] separated a fraction from a vitamin A concentrate, by treatment with fullers' earth, which gave an absorption band in the antimony trichloride reaction at about 580 mμ, as against 620 mμ for the vitamin itself A year later von Euler, Karrer and Zubrys [28] separated a similar substance by submitting a vitamin A concentrate to chromatographic adsorption on calcium hydroxide In the antimony trichloride reaction an absorption band appeared first at 580 mμ, but was replaced by a band at 620 mμ on standing The substance was presumed to be a carotenoid, and was given the name "hepaxanthin" The biological activity, if any, was very low Castle *et al* [20], in making a detailed study of the products obtainable by chromatography from vitamin A concentrates, separated a fraction which they identified as "hepaxanthin". In addition to the band at 580 mμ in the antimony trichloride reaction it had absorption at 270–280 mμ in the ultraviolet Another fraction more strongly adsorbed, was thought to be the same material as had been described by Van Eekelen and his colleagues Both fractions were considered to be oxidation products of the vitamin, with hepaxanthin at an earlier stage of oxidation than Van Eekelen's material With our present knowledge of the oxidative power of fullers' earth this suggestion seems very reasonable

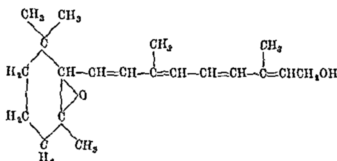
Le Page and Pett [29] observed an absorption band at 275 mμ after treating shark-liver oil with hydrogen peroxide In a more detailed chemical study Karrer and Jucker [30] oxidised synthetic vitamin A with monoperphthalic acid The epoxide so obtained had an absorption max 275 mμ, with $E^{1\%}_{1\,cm}$ = 450 and resembled hepaxanthin, as previously described, in giving a band in the antimony trichloride reaction first at 580 mμ and later at 620 mμ Karrer was unable to decide whether the oxygen atom was attached to the ionone ring, or to the two atoms in the side chain nearest to the ionone ring In addition to the epoxide another product was isolated which had $E^{1\%}_{1\,cm}$ = 850 at 339 mμ and which gave a band at 575 mμ in the antimony trichloride reaction Treatment of the epoxide with hydrogen chloride gave two further derivatives One had absorption bands at 334, 350 and 368 mμ The other resembled substance X, later described by Meunier [23], in having spectroscopic properties similar to vitamin A but no biological activity

The spectroscopic changes which have been reported during the atmospheric oxidation of vitamin A usually include the formation of a band at about 270–280 mμ It seems probable, therefore, that the formation of the

References p 126

epoxide is included in the various stages of oxidation Halpern[31] reported that when fish-liver oils were oxidised in air at 75 °C bands developed at 235 and 280 mμ The first position is suggestive of oxidation in the glyceride fraction Bolomey[32] noticed bands at 274–275, 284–286, 294–299 and 310–312 mμ in solutions of vitamin A acetate in triacetin which were aerated or stored Groot[33] sometimes observed bands at 220, 273 and 310 mμ when vitamin A was made up in very dilute alcoholic solution Solutions exposed to diffuse daylight developed incipient bands at 273, 280 and 310 mμ

From these observations it seems that the first derivative of vitamin A during oxidation absorbs at 310 mμ, and that "hepaxanthin" is a later product It may be significant that preparations of vitamin A acetate, as used for the International Standard, always show an inflection at 310 mμ



Probable formula of hepaxanthin (vitamin A epoxide?)

Subvitamin A. This substance, which appears to be another oxidation product of vitamin A_1, or possible of vitamin A_2 was found by Embree and Shantz[34] in shark-liver oil Greater solubility in 83% ethyl alcohol and stronger adsorption on alumina allowed its separation from vitamin A_1 The richest concentrates obtained had an absorption band at 290 mμ with $E^{1\%}_{1\,cm} = 150$ In the antimony trichloride reaction the absorption band was at 617 mμ, with $E^{1\%}_{1\,cm} = 310$ Dehydration with hydrogen chloride gave bands at 332, 348 and 367 mμ, as compared with 351, 371 and 392 mμ for vitamin A No biological activity could be detected

Substances Z_1 and Z_2 Meunier and his colleagues[35, 36] obtained oxidation products, by treating vitamin A in light petroleum with vanadium oxide, which were claimed to be toxic to rats Z_1 could be extracted from the reaction products in light petroleum by means of aqueous acetic acid It had bands at 340 mμ in the ultraviolet and at 545 in the antimony trichloride reaction, and was thought to be a derivative of retinene with two hydroxyls on the terminal carbon of the side chain and one hydroxyl near the centre of the chain Z_2 had bands at 255 and 290 mμ in the ultraviolet and at 485 mμ with $SbCl_3$ It was thought to be a derivative of

retinene with two hydroxyls group present near the centre of the side chain An equilibrium between the two forms was assumed

Kitol The paper by Embree and Shantz on subvitamin A was accompanied by a further communication [37] in which they described another new congener of vitamin A Kitol (Greek kitos = whale) was obtained by adsorbing the residue left after the distillation of whale liver oil on a column of aluminium oxide It had an absorption band at 290 mμ, and in the antimony trichloride reaction gave a red colour with bands at 428, 505 and 580 mμ The elementary analysis corresponded nearly to $C_{40}H_{60}O_2$ and determinations of molecular weight were in good agreement with this formula Eight double bonds and two hydroxyls were found There was slight optical activity, with [α] at 25 °C and 546 1 mμ in chloroform = 1 35 ° Unlike subvitamin A the solubility in light petroleum was greater than in 83% ethyl alcohol

In biological tests kitol was inactive, but on heating to over 200 °C it broke down to give vitamin A Thus an absorption band appeared at 328 mμ, a blue colour was given with antimony trichloride with a maximum at 617 mμ and biological activity was developed The production of vitamin A from kitol was not affected by previous treatment with hydrogen chloride, but was prevented by treatment with antimony trichloride Small amounts of kitol were also detected in the liver of the shark and of the lamb

Later Clough *et al* [38] crystallised pure kitol from methyl alcohol in the form of elongated prisms which melted at 88–90 °C For kitol itself $E^{1\%}_{1\,cm}$ at 290 mμ was 707 and for the palmitate 379 The exact structure of kitol is still unknown Under the best conditions of distillation each molecule of the palmitate gives only 0 75 molecule of vitamin A

The discovery of kitol recalled work by Pritchard, Wilkinson, Edisbury and Morton [39] in which a fraction with similar spectroscopic properties was separated from a concentrate made from ' mammalian" liver oil We can now suspect that the mammal in question was a whale

Di vitamin A ether Another condensation product of the vitamin formed by an ether linkage between two of its molecules has been reported by Meunier and Vinet [40] When the blue compound formed by the adsorption of vitamin A on acid clay was eluted it gave yellow material from which red crystals were obtained In the ultraviolet maxima at 330 and 430 mμ were observed and in the antimony trichloride reaction at 589 mμ The molecular weight of 504 suggested a condensation product It was considered to be identical with yellow coloured derivatives of vitamin A which had previously been reported by Ender [41], Castle *et al* [20], and Holmes and Corbet [42]

Homovitamin A This artificial homologue differs from vitamin A in having its side chain extended by an extra CH_2 group

References p 126

before the terminal hydroxyl group It was synthesised in the form of its ethyl ether by Milas *et al.*[43] The absorption maximum varied from 321 to 328 mμ according to the method of preparation 5-Dehydro-homovitamin A ethyl ether, in which one of the double bonds in the side chain was replaced by a triple bond, had a band at 321 mμ and was found to have slight activity in biological tests

VITAMIN A_2 AND ITS DERIVATIVES

Vitamin A_2 The highest concentrations of this substance, which differs from vitamin A_1 in having an extra conjugated double bond in the β-ionone ring, are found in the livers of fresh-water fishes Its discovery emerged eventually as a sequel to early observations that bands at positions other than 620 mμ were occasionally produced in the antimony trichloride reaction by fish liver oils Thus in 1931 Heilbron, Gillam and Morton[44] noticed that in addition to the usual subsidiary maximum at 583 mμ others were sometimes present at 635, 645, 656, 680 and 693 mμ Six years later the livers of certain Russian fresh-water fish were examined by Lederer and Rosanova[45], who found much higher concentrations of the 690 mμ chromogen than had previously been reported Wald[46], approaching the problem from a different angle found that a band at 696 mμ was produced in the antimony trichloride reaction by extracts of the bleached retinas of fresh-water fishes He assumed that this chromogen played a role parallel to vitamin A in vision by producing a modified form of visual purple

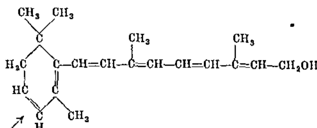
The description "vitamin A_2" appears first to have been applied by Edisbury, Morton and Simpkins[47] Extracts of the liver and viscera of brown trout gave a maximum at 693 mμ in the antimony trichloride reaction, and without the reagent bands were seen at 470, 350 and 287 mμ In an extensive study of extracts from Russian fresh water fishes, Gillam, Heilbron, Jones and Lederer[48] confirmed the predominence of the 693 chromogen, and of the main band at 340–350 mμ in the ultraviolet From the absorption spectrum the presence of 6 conjugated double bonds was assumed, and the molecule was thought at the time to have two carbon atoms more than vitamin A_1 in its side chain Preliminary biological tests suggested that vitamin A_2 could promote growth in deficient rats The interpretation of biological tests, however, was complicated by the presence of substantial amounts of vitamin A_1 even in the best concentrates of vitamin A_2 which were then available

Gray[49] found little difference between the distillation ranges of vitamin A_1 and A_2, and concluded that both molecules contain 20 carbon atoms Karrer and Bretscher[50] prepared a concentrate from the liver of pike, which were collected during the winter when the proportion of vitamin A_2

to vitamin A_1 is highest $E^{1\%}_{1\,cm}$ was 1450 at 345 mμ in the ultraviolet, and 2300 at 695 mμ in the antimony trichloride reaction. About 2–3% of vitamin A_1 was present, and when allowances were made for this impurity the results of biological tests suggested that vitamin A_2 had only 5–7% of the biological activity of vitamin A_1. From chemical experiments it was concluded that vitamin A_2 was isomeric with vitamin A_1, and that it had an open chain structure similar to that of lycopene. The unconfirmed claim by Meunier [51] that lycopene is converted to retinene$_2$ on adsorption on manganese dioxide appeared to support this view.

Biological tests by Jensen *et al.* [52] made on a concentrate of vitamin A_2 which appeared to be free of vitamin A_1, again indicated only low activity. Later Shantz [53] obtained a specimen of virtually pure vitamin A_2 which had $E^{1\%}_{1\,cm}$ at 352 = 1460, at 287 mμ = 820, and at 693 mμ (with $SbCl_3$) = 4100. Greater biological activity, equal to 40% of that of vitamin A_1 was now reported [54] Nearly the same spectroscopic values were found by Morton and his colleagues [55, 56] in experiments which gave further proof that complete purity had been obtained. Although vitamin A_1 and A_2 could not be separated by chromatography the same difficulty was not involved with their aldehydes A rich vitamin A_2 concentrate was therefore oxidised with manganese dioxide retinene$_2$ was separated by chromatography and crystallised, and vitamin A_2 was regained by reduction with lithium aluminium hydride When so purified it had $E^{1\%}_{1\,cm}$ in ethanol at 351 = 1410, at 286 mμ = 698, and at 693 (with $SbCl_3$) = 3700

Confirmation of the vitamin A_2 as the 3-dehydro derivative of vitamin A_1 was finally obtained by Farrer, Hamlet, Henbest and Jones [57]. An extra double bond was introduced into the β-ionone ring of the methyl ester of vitamin A acid by bromination with N-bromo-succinimide followed by dehydrobromination with 4-phenyl morpholine Treatment with lithium aluminium hydride then gave a product which agreed closely in its spectroscopic properties with natural vitamin A_2 Biological tests indicated 30% of the biological activity of vitamin A_1



Vitamin A_2, $C_{20}H_{28}O$

References p 126

Derivatives of vitamin A_2 It would appear that a whole set of derivatives could be prepared from vitamin A_2 to correspond to those which have been described for vitamin A_1 Relatively few of these derivatives have so far been isolated or synthesised Adequate descriptions have been given, however, of the aldehyde and anhydro derivative

Crystalline retinene$_2$ as prepared by Morton and his colleagues[55, 56] had m p 77–78 °C, $E^{1\%}_{1\,cm}$ at 385 mμ = 1490 and at 730–740 mμ (with $SbCl_3$) = 4000 Its 2,4 dinitrophenylhydrazone and semicarbazone were also obtained in crystalline form

Anhydrovitamin A_2 was found by the same workers to have absorption bands in ethanol at about 350, 370 and 390 mμ, with an inflection at 330 mμ In the antimony trichloride test the maximum was at 693 mμ It will be recalled that anhydrovitamin A_1 has similar absorption in the ultraviolet, but differs considerably in having its band at 620 mμ in the antimony trichloride reaction

Before leaving the subject of vitamin A congeners we may do well to reflect on the difficulties involved in their investigation, and on the oppor tunities for further investigation, perhaps not always very profitable, in this field We have mentioned that there are several possible *cis trans* isomers of vitamin A_1 If we overlook questions of steric hindrance we may also, at least in theory, have groups of aldehydes acids dehydro com pounds epoxides furanoid compounds, and various combinations such as aldehyde epoxides These sets of compounds will be duplicated for vitamin A_2 and the number of derivatives may be increased by violent chemical action which disrupts the carbon skeleton All these compounds will be unstable and will tend to change on brief exposure to air, or even on chro matographic adsorption during attempts at purification At the moment it seems over-optimistic to hope that the whole network of possible derivatives will ever be described in full detail Great credit is due to the investigator whose patient and laborious work has made possible the progress whic has already been accomplished

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PART IV

THE COMPARATIVE BIOCHEMISTRY AND NATURAL HISTORY OF THE CAROTENOIDS AND VITAMIN A

CHAPTER 12

Plants and Micro-Organisms

As a prelude to a specialised study of vitamin A in the higher animals it is interesting [illegible] whole realm of nature, and to see how the vitamin, ar[illegible] different fo[illegible]. [illegible] cluding many bacteria and fungi, can thrive in the absence of carotenoids, most such organisms are concerned in their synthesis or metabolism. Thus carotenoids are synthesised by green plants, and also by certain yeasts, bacteria and fungi When these plants are eaten by animals the carotenoids may be rejected or destroyed, may be stored in unchanged form, or may be converted into vitamin A or into predominantly animal pigments such as astaxanthin. A full account of all the bewildering range of possibilities is beyond the scope of this book, and the reader interested in the comparative biochemistry of the carotenoids should refer to the excellent treatise on this topic by Goodwin [1]. Much valuable information on the distribution of carotenoids is also available in Karrer's book [2] In the present work we cannot hope to discuss more than a few points which seem to have special significance in a fascinating field which has not yet been fully explored

Carotenoids in Terrestrial Plants

Green foliage The leaves of all green terrestrial plants contain carotenes, with the β-form normally predominating, and hydroxylated xanthophylls. According to Goodwin the ratio of xanthophylls to carotenes varies from 4–9 : 1 in most species, but may rise to 15 : 1 in some Alpine plants In specimens of fresh grass Moon found lower ratios between 2 : 1 and 3 : 1, with 2 2 : 1 most common [3] The leaves of etiolated plants contain both xanthophyll and carotene, which presumably provide the trigger mechanism for starting photosynthesis when the plant is exposed to light.

The carotenoids have their absorption maxima in the blue region. As far

References p 137

as can be judged from their properties after isolation they will readily absorb oxygen It is temping to conclude that they play some part in the formation and metabolism of chlorophyll as suggested in 1913 by Willstatter and Stoll[4] More modern knowledge of the similarity of the carbon skeletons in the phytol fraction of the chlorophyll molecule and in the carotenoids must greatly strengthen this hypothesis (See Formula Group 8, Chap 9)

Willstatter suggested that carotene and xanthophyll might interact with chlorophyll A and chlorophyll B in a coupled redox system There is no clear evidence, however, that carotenes are reversibly oxidised to xanthophylls in plant or animal tissues Neither is evidence available that phytol may be derived by the saturation, ring opening and hydrolytic splitting of the carotene molecule, or that carotene can be derived from phytol by changes in the reverse direction In spite of our detailed knowledge of the distribution of carotenoid pigments in plants their role in photosynthesis and metabolism, a matter of fundamental importance for the maintenance of life on this planet remains elusive

Yellow plant tissues Although most green tissues tend to contain a constant excess of xanthophyll over carotene this relationship does not apply to yellow plant tissues Different carotenoids predominate in the yellow tissues of various plants Thus the carrot root is mainly coloured by β carotene, with a small proportion of the α-form The coloured part of the palm fruit also contains mainly carotene, but with a higher proportion of the α form than in the carrot On the other hand xanthophylls predominate in many flowers and fruits The main pigment of the petals of *Helenium autumnale* is lutein dipalmitate, while the fruit of the *Physalis alkekengi* contains zeaxanthin dipalmitate Flowers also frequently contain carotenoid derivatives which are not present in the corresponding foliage such as epoxides and *cis*-isomers

Speculation on the biochemical role of carotenoids in these yellow flowers and fruit is made difficult by doubts as to how far they are really essential for the welfare of the plant We may perhaps picture the carotenes and xanthophylls as having an essential role in promoting and maintaining photosynthesis This role would demand that their proportions should be kept constant In tissues not directly concerned with photosynthesis, however, the same constant proportion would no longer be enforced Thus in tissues collecting reserve materials required for reproduction, or for the survival of the plant during a period unsuitable for synthesis, carotenoid metabolism might be released from the control exerted by its association with chlorophyll Under these conditions carotenoids might become waste products, or be used in new roles The disturbance of the usual xanthophyll to

carotene ratio moreover, might be accompanied by the formation of derivatives of these pigments which are not found in green foliage

In support of this view we may note that varieties of the same species may often be equally healthy plants whether their flowers, fruits or storage tissues contain high concentrations of carotenoids or not Thus crocuses may survive equally well with white or yellow petals, while maize seeds may be a deep yellow or almost white. Dark and Booth [5] found about a 5 fold range in the carotene contents of ordinary yellow and red carrot roots, and special white varieties are cultivated The root of the parsnip, a vegetable which closely resembles the carrot in its habits, contains 100 times less carotene If we are to assume that carotenoids play an important role in the metabolism of the more highly coloured forms of these plants we must also conclude that alternative metabolic routes are available in those which are less highly coloured

In ripening fruits the disappearance of chlorophyll is often accompanied by a rise in the concentration of carotenoids, and by an increase in the ratio between carotenes and xanthophylls Maturity in the tomato is indicated by the disappearance of chlorophyll and the appearance of lycopene Kuhn and Grundmann [6] found, however, that the amount of lycopene formed was 8 times that which would have been expected from the amount of phytol in the disappearing chlorophyll If the lycopene is derived from phytol, therefore sources other than chlorophyll must be tapped

More recent studies by Porter and Lincoln [7] have revealed the presence in tomato extracts of an interesting series of substances which lose their yellow colour and gain blue fluorescence under ultraviolet irradiation as they become more saturated The series may best be remembered as starting from lycopene, with 13 double bonds and proceeding through tetrahydrolycopene (11) to 'ζ carotene' (9) phytofluene (7), phytoene (5) and tetrahydro phytoene (3) The last member could presumably be derived from the condensation of two molecules of phytol Porter and Lincoln visualised the formation of lycopene from more saturated members of the series by progressive stages each involving the loss of four hydrogen atoms They have shown that the relative proportions of each substance in the ripened plant depends on genealogical factors While this influence cannot be questioned Goodwin remains uncertain whether the series is built up towards the formation of lycopene, or whether lycopene is formed first and its more saturated derivatives afterwards All these findings suggest caution against the conclusion that the presence of a high concentration of carotenoid in a particular site must imply that its action in the site concerned is correspondingly intense It may be argued that if mechanisms for the disposal of unwanted carotenoids are not available they must be deposited somewhere in the

References p 137

tissues Sometimes these deposits, as in flowers, may attract insects or birds, or serve some other useful purpose

Carotenoids in Micro-Organisms and Fungi

Bacteria Although numerous bacteria have already been examined for carotenoids it remains difficult to decide how many of the more common species contain significant quantities Workers have naturally concentrated their studies on those bacteria which are particularly rich in carotenoids Often the carotenoids have been identified qualitatively without estimation of the quantities present

Goodwin comments that β carotene is present with much less regularity in bacteria than it is in plants The common form of xanthophyll or lutein, as found in green plants, is almost invariably absent Most carotenogenic bacteria, however, produce special carotenoids, which are often in the xanthophyll group Sometimes methyl ethers of xanthophylls are formed

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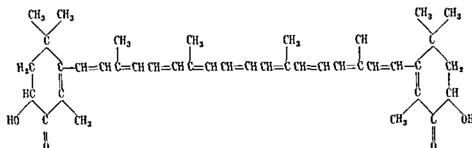
pyogenus aureus which is frequently found in pyogenic infection in man, has been reported *to owe its yellow colour to δ carotene, zeaxanthin and* rubixanthin (3-hydroxy γ carotene)[10, 11, 12] Other bacterial carotenoids, which can be mentioned only by name, are corralin, rhodopin, rhodoviolascin, rhodopurpurin, rhodovibrin, flavorhodene, sarcinene, sarcinoxanthin, rhodoxanthin, α and β-bacteriopurpurins and crysophlein

It is clear that some bacteria produce considerable quantities of carotenoids It seems equally evident, however, that the organisms commonly present in the intestines of animals make no significant contribution to the requirement for provitamins Although McGillivray[13] has claimed that carotene is synthesised in the intestines of the sheep there has been no authentic instance in any animal of vitamin A deficiency being averted by the intervention of the intestinal micro organisms

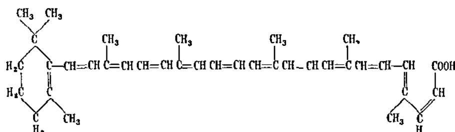
Moulds and fungi The inconsistent distribution of carotenoids typical of the bacteria is repeated in the moulds and fungi. Goodwin lists some 60 fungi from which carotenoids are absent Other species, some of them edible, synthesise large amounts and appear brightly coloured Again β carotene is by no means universal, and the common form of plant xanthophyll is absent Acidic pigments resembling astaxanthin, which is important in some animal species, are often present

Of the two forms of anascoporogenous yeasts the Rhodotorulaceae contain carotenoids but the Torulopsidaceae do not The pigments *found in strains* of *Rhodotorula rubra* are torularhodin, m p 201–203 °C which is an acidic

pigment, torulene, m p 185 °C which is believed to be 3,3 dimethoxy-γ-carotene, and small amounts of β carotene. In 1 g of a dried culture of *R. sanniei* Fromageot and Tschany [14] found no less than 2900 μg of torularhodin, 143 μg of torulene and 10 μg of β-carotene. Since Karrer and Rutschmann [15] have reported that torularhodin is effective as a provitamin A it must be inferred that torula yeasts have high vitamin A activity In this respect they must differ from the brewers' yeast cultured from *Saccharomyces cerevisiae*, which is often included in dried form in basal diets deficient in vitamin A



Astaxanthin $C_{40}H_{52}O_4$



Torularhodin, $C_{37}H_{48}O_2$

Phycomyces

Another organism which has been extensively studied is the microscopic fungus *Phycomyces blakesleeanus* The main pigment produced is β-carotene, but α carotene is also produced and traces of lycopene and other pigments have been detected Goodwin and his colleagues [16] have followed up work by Schopfer [17] on the biochemical factors which influence the formation of carotenoids Carotene may be synthesised from glucose, acetate or amino acids In the absence of glucose synthesis is more rapid when the culture medium contains valine than when it contains asparagine, but is even more rapid with leucine. Valine presumably gives rise to a derivative with the *iso*pentane carbon skeleton in the course of metabolism by *Phycomyces*, and leucine may follow a similar path after the loss of a carbon atom by decarboxylation When it is grown under favourable conditions the fungus may contain as much as 150 mg of pigment per 100 g dry

References p 137

weight of the mycelium, but when the allowance of carbohydrate is restricted the concentration reaches only 20–30 mg per 100 g

Carotenoids in Aquatic Plants and Diatoms

Marine algae Some algae closely resemble the terrestrial plants in their carotenoid contents, but others diverge widely Thus the main pigments in many of the Chlorophyceae, including the familiar green seaweeds *Enteromorpha intestinalis* and *E. compressa*, are β carotene and xanthophylls An interesting exception has been found in *Trentepohlia aurea*, which contains β-carotene by itself in high concentration Certain other green algae, such as *Haematococcus pluvialis*, depart from the general patterns by containing astaxanthin[18] This finding was highly important in showing that the distribution of astaxanthin is not limited to animals

In the brown Phaeophyceae, the largest class of algae and the most prolific in distribution, β-carotene is present, but always in much smaller concentration than the characteristic fucoxanthin According to Heilbron and Phipers[19] this pigment is an open chained, tetrahydroxy, diketo carotenoid

In the red Rhodophyceae carotene is always present, but these algae have no characteristic carotenoid corresponding to fucoxanthin in the brown weeds Only one member, *Polysiphonia nigrescens*, overlaps with the Phaeophyceae in containing fucoxanthin

The Euglenineae, minute flagellate algae which are very suitable for experimental culture are interesting as another example of the presence of astaxanthin outside the class of invertebrate animals Their astaxanthin was first isolated under the name of euglenarhodone[20]

Diatoms These minute organisms are important in the ultimate sources of carotenoids, and indeed food in general, for higher forms of marine life *Nitzschia closterium* in particular was studied in early work on the mode of formation of vitamin A Diatoms all contain β carotene, but opinions differ as to whether they contain the same xanthophyll derivatives Fucoxanthin is present but not astaxanthin

Enzymic Destruction of Carotenoids

Lipoxidase The instability of isolated carotenoids in atmospheric oxygen is one of their best known properties We have little information, however, on their fate in vegetable tissues Although the change from carotene to vitamin A can be carried out in the laboratory no evidence has yet been obtained to show that the vitamin itself is ever present in vegetable tissues

Sumner and Dounce[21] demonstrated that solutions of carotene in oil

were bleached when they were incubated with enzyme systems prepared from soya beans and certain other legumes The presence of unsaturated fat is necessary for the working of the enzyme which presumably catalyses the oxidation of the fat at its double linkages and then further catalyses the action between the oxidised fat and the pigment Carotenoids other than carotene may also be oxidised by the same mechanism

CONCLUSIONS

Variations in carotenoid distribution in different vegetable species

The large number of carotenoid pigments and their derivatives and the apparent fortuity of their distribution in many tissues makes it difficult to reach any broad generalisation about their distribution and behaviour in vegetable organisms

We have seen however that the highest degree of uniformity is found in green plant tissues which always contain carotenes together with larger quantities of xanthophylls In yellow plant tissues the relationship between carotenes and xanthophyll is usually broken with either carotene or xanthophyll predominating Epoxides *cis trans* isomerides and derivatives which are characteristic of the particular plant make their appearance and greatly complicate the picture Even greater differences and distinctions are found between the micro organisms carotenoids may be completely absent or present in surprisingly high concentrations

In green seaweeds the pigments often resemble those in land plants but fucoxanthin distinguishes brown weeds Astaxanthin typical of invertebrate animals is seldom present in vegetable tissues It has been detected however in some species of algae

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CHAPTER 13

Animals

In the preceding chapter we have seen how vegetable organisms differ greatly in both the ... of carotenoids which they synthesise and in the chemica...
can remain that ...
vegetable sources ...
applied without exception to all animals As ...
most animals do not admit to their tissues all the carotenoids which are present in their diet It must be assumed that some pigments are less readily absorbed than others from the intestines or that they are degraded into colourless products by methods which are not yet understood Other pigments may pass unchanged into the tissues or may be converted into derivatives such as vitamin A and astaxanthin which are characteristic of animals

The mechanisms which control the absorption of some pigments and the rejection or m... of species and sometimes even of variety ... Thus the fat of cows given grass will be coloured with carotene while in chickens the fat ... In some breeds of chickens the xantho...

metabolism ...
containing carotene are usually much greater than ...
guinea pigs given the same diet

Between higher and lower animals moreover an even greater distinction can be drawn The invertebrates with a few exceptions do not convert carotenes to vitamin A Some species however can change carotene and possibly other carotenoids into astaxanthin On the other hand the higher animals make vitamin A but their tissues seldom contain astaxanthin

Invertebrates

Land invertebrates The occurrence of carotenoids in terrestrial i does not seem to have been fully explor points out that some species, including the clothes moth and the can subsist on diets free from carotenoids In some Lepidopte carotenoids are present in the haemolymph and hypodermis ported that female silkworms, *Bombyx mori*, contain caroteno males are colourless

Extensive studies on locusts by Goodwin and Srisukh [2] have the haemolymph contains carotene, but the integument astaxa win [3] found only *β* carotene in newly laid locust eggs, but as vanced the carotene concentration fell and astaxanthin appear crucial observations the ability of some animals to make astax other carotenoids was placed beyond all doubt

Lower marine invertebrates Carotenoids are widely distribute starfishes, sea anemones, sea urch and gastropods High concentrations are often attained, and th correspondingly striking in appearance Thus the sex glands of scallop are very highly coloured The usual forms of carotenes phylls are often present, but sometimes characteristic derivativ Thus echinenone the main pigment present in sea-urchin gonad by Goodwin and Taha [4] to be 4-keto *β* carotene Lederer and M that it was effective as provitamin A

At one time it appeared that echinenone had the distinction of vitamin restricted in its distribution to animal species Goodwi however consider that it is identical with myxoxanthin which from *Oscillatoria rubescens* by Heilbron and Lythgoe [6] and also w isolated from *Aphanizomenon flos aquae* by Tischer [7] It remain whether sea urchins and other marine invertebrates such as lim their echinenone directly from algae or by modifying *β*-carote other common pigment

Not more than traces of vitamin A have been reported in a animals Substantial quantities have been detected, however, in the much more highly developed cephalopods, which include th and squids [8]

Crustacea The characteristic carotenoid in crabs lobsters, shri lated arthropods is astaxanthin, which is often conc the integument *β*-Carotene and xanthophyll are also usually pre

In some Crustacea the reddish brown or yellow colour of the in consistent with the presence of unmodified astaxanthin The car

lobster, however, contains astaxanthin combined with protein in the form of a blue coloured complex When the lobster is boiled the complex is broken down and the astaxanthin is oxidised into the artifact astacin, or 3 4, 3',4'-tetraketo β carotene, with a change in colour to bright red According to Kuhn and Lederer [9] the green pigment of the lobster eggs is a similar complex It is perhaps relevant that when astaxanthin is treated with potassium butoxide in the absence of air it turns blue, presumably owing to the enolis ation of the hydroxyl groups followed by the formation of a potassium salt [10]

In a book about vitamin A however, the Crustacea are of special importance in providing exceptions to the general rule that vitamin A is found only in vertebrates According to an early report by Wald [8] vitamin A is present in the fresh water shrimp *Gammarus virilis* Neilands [11] found large amounts of vitamin A in the eyes and hepatopancreas of the common lobster, *Homarus vulgaris* Kon and Thompson [12, 13] found vitamin A, but only minute amounts of β carotene in the exoskeletons and particularly in the eyes, of the shrimps or krill that provide the nutriment of some species of whales

It is interesting that although locusts resemble the crustaceans in having astaxanthin as their characteristic pigment there is no evidence of the presence of vitamin A in any part of their bodies

Vertebrates

Fish Some fish such as the common gold fish have their skins highly coloured with carotenoids, which are usually xanthophylls In others carotenoids may be absent or at least much less prominent The salmon has little evidence of carotenoid pigmentation in its skin, but its muscles are coloured pink with astaxanthin

In the livers of marine fish vitamin A_1 predominates The concentrations attained vary greatly according to species (Appendix Table 64-72) The livers of fresh water fish usually also contain vitamin A_1, which is generally mixed with substantial amounts of the more highly unsaturated vitamin A_2 With the exception of a few oils coloured with astaxanthin the liver and body oils of most common fish are faintly coloured

The livers of some sharks and particularly the soup-fin shark contain very large amounts of vitamin A This finding, however, does not apply to all species Thus the liver of the basking shark, which is found off the coast of Ireland, is a very poor source of the vitamin Possibly the synthesis of squalene with its molecule based on six *iso*pentane units, takes precedence over the formation of vitamin A in this animal

Amphibia Carotenoids have long been known to occur in the skins and organs of many species of frogs and toads Rand [14] demonstrated the presence of carotenoids in the skin, liver, lungs ovaries, testes and fat

References p 147

bodies of frogs both in summer and in winter Zechmeister and Tuzson[15] identified the pigments concerned as α and β carotenes xanthophyll and zeaxanthin Morton and Rosen[16] found that vitamin A was concentrated in the liver and eyes of the frog *Rana temporaria* and that carotenoids were present in almost all tissues but with the highest concentration in the skin and ovaries Xanthophyll always predominated over carotene with a ratio of about 3 1 in its favour in the skin and 10 1 in the ovaries

In two species of newts *Triturus cristata* and *T carnifex* the livers and ovaries were found by Collins Love and Morton[17] to contain both vitamins A_1 and A_2 Carotenoids were also mainly concentrated in the liver with the carotene fraction in about two fold excess over the xanthophyll The same workers[18] also studied the axolotl *Amblystoma tigrinum* both in the form of the sexually mature aquatic larva and as the terrestrial adult The animals were kept on various diets which differed in their contents of carotene and preformed vitamin A They remained healthy even when they were given diets which contained no vitamin A but if the preformed vitamin was given it was absorbed and stored in the liver On the other hand there was no evidence that carotene could be converted to vitamin A Only a small fraction of the dietary intake of pigment was stored unchanged in the liver

Reptiles Information on the carotenoid metabolism of reptiles is sparse Manunta[19] found xanthophyll esters together with small amounts of free xanthophyll and a little carotene in the skin of the African chameleon *Lacerta viridis* Villela and Prado[20] found xanthophyll but not carotene in the blood of two out of four species of Brazilian snakes Carotenoids have been found in large amounts in the liver of the tortoise *Testudo graeca*[21]

Gillam[22] found that the livers of the giant monitor *Varanus salvator* (a large lizard) and python *Python reticulatus* were far richer in vitamin A than the livers of any other land animals which he examined The vitamin was also found in an alligator s liver but only in moderate concentration Further evidence of the high vitamin reserves of monitors was obtained by Jensen and With[23] in examining a specimen of *Varanus comodensis* High values were also found for the viper *Vipera berus*

Birds In general birds absorb xanthophylls into their bodies but convert carotenes into vitamin A At the most only traces of unchanged carotenes are passed into the tissues

Xanthophyll is included in egg yolk in the free form but it is esterified before storage in the skin or shanks Although it is difficult to quote evidence from the literature common experience indicates that the details of xanthophyll metabolism vary in different species of birds and even in different tissues in the same bird As a familiar instance it is clear that the egg yolk of

hens and ducks are both yellow, but that the hen has usually very much yellower body fat than the duck Some breeds of chickens may combine strongly yellow body fat with colourless shanks

Great differences are evident, moreover, in the extent to which various species of birds employ carotenoids for colouring their plumage According to Brockmann and Völker [24] the yellow feathers of birds owe their colour to a mixture of ordinary xanthophylls and canaryxanthophyll The latter pigment, as its name suggests, predominates in the feathers of the canary The flamingo, and a few other birds, are interesting in having their feathers and fat coloured with phoenicotterin, which is presumably identical with astaxanthin [25]

Carotenoids in mammals Vitamin A is found in all mammals consuming their natural diets, but there are remarkable differences in the extent to which unchanged carotenoids are passed into the fat deposits, and other tissues

Many animals have virtually colourless body fat, although it is not safe to deny that traces of pigment may be present when the diet is rich in carotenoids The sheep goat, pig, dog, cat, rat, guinea pig and most rabbits are familiar members of this large group

In man the blood plasma and body fat are usually golden yellow through the presence of carotenoids β Carotene is usually the chief component, but substantial amounts of xanthophyll, lycopene and another unidentified carotenoid have been reported In the cow β carotene is also the predominating pigment The yellow coloration is usually much more intense than in the human and the proportion of pigments other than carotene is much smaller In animals of the Guernsey and Jersey breeds the degree of pigmentation is particularly intense In the opposite direction the pigmentation of Indian buffaloes appears to be much lower than cows, and the milk fat is sometimes colourless [26]

Carotene also predominates in the horse, but the yellow colour of the plasma of this animal appears to be due in part to bilirubin [27, 28, 29] The only instance of the selective storage of xanthophylls by mammals has been observed as an unusual recessive characteristic in rabbits [30, 31]

Vitamin A in mammals The quantities of vitamin A accumulated by mammals, and stored in their livers, vary widely between species, and also between individuals in the same species These variations within the species are doubtless not confined to mammals, but the wealth of data which is available on mammals has made the wide ranges of individual values more apparent

Age is one factor which influences the vitamin A contents, since the livers of most newly born animals contain only small amounts of the vitamin Sex

References p 147

is also concerned, since females tend to accumulate higher stores of vitamin than males (see Chap 35) There may also be seasonal variations The most important factor, however, is usually the food supply Thus it is possible to maintain rats in good general health with liver reserves between 0 and 10,000 i u per g simply by varying the dietary intake of vitamin Comparisons between different animals, therefore, are only significant when they are subsisting on a diet which is characteristic for the species The values for the liver reserves of common mammals given in Table 8 are typical, in the author's opinion, for animals living under natural conditions, or under the most usual conditions of farm husbandry Values for some non mammals are included for comparison

must often ingest provitamins in amounts which would rapidly saturate their livers if they were efficiently converted to vitamin A In practice however, the rate of wastage, through failure in absorption and other causes, is very high In herbivorous animals receiving the same food, species become the limiting factor

Thus in both sheep and cows which are fed upon good green pasture the amount of vitamin A which is accumulated in the liver usually corresponds to an amount of carotene which could be eaten in a very short period of grazing, possibly in a few hours But even if absorption and conversion are inefficient in both species the sheep usually accumulates about 600 i u per g of liver, as compared with only 150 i u in the cow This difference may perhaps be related to a greater efficiency of the conversion of carotene to vitamin A in the intestinal wall of the sheep, as evidenced by the absence of yellow colour in its blood plasma and body fat

White body fat, however, does not in itself ensure that a species of animal will be able to accumulate large stores of vitamin A when given a diet containing provitamins Chevallier and Choron[32], and later Bentley and Morgan[33] found that most guinea pigs, in spite of their colourless fat, accumulate notably low reserves of vitamin A when they are given a diet containing green vegetables They are quite efficient however, in storing preformed vitamin A In the author's own experience the livers of guinea pigs given a mixed diet including greens usually contained only about 10 i u per g, as compared with 150 i u in rabbits given the same diet

The vitamin A ladder in land animals

On land the ladders which are followed in the transference of vitamin A from lower species to higher species are usually relatively short Thus it is normal for humans to derive much of their vitamin A by converting carotene ob-

ich may be consumed either by their own young or by man Eventually
herbivora are usually killed by man or by carnivorous animals and their

man

It is perhaps rather surprising that the vitamin A reserves reported for
edatory mammals such as lions and tigers have not been particularly
gh and sometimes decidedly low Possibly the data have been misguiding
referring mainly to animals kept in captivity which may have been fed
tirely on skeletal muscle The very high reserves for carnivorous reptiles
hich swallow their victims whole are in striking contrast

TABLE 8

PICAL VALUES FOR THE HEPATIC RESERVES OF VITAMIN A IN DIFFERENT SPECIES
N B IN EVERY SPECIES WIDE INDIVIDUAL VARIATIONS ARE TO BE EXPECTED

Species	*Vitamin A i u /g liver*	*Species*	*Vitamin A i u /g liver*
Guinea pig	10	Horse	600
Frog	30	Hen	900
Pig	100	Greenland seal	2 000
Dog	100	Cod fish	2 000
Vole	100	Python	3 000
Cow	150	Giant monitor	4 000
Rabbit	150	Sperm whale	4 500
Rat	250	Halibut	10 000
Human	300	Bearded seal	13 000
Fox	500	Polar bear	20 000
Sheep	600	Soup fin shark	50 000

Vitamin A ladder in marine animals

The transfer of vitamin A between animals in the sea usually runs through more stages than on land Minute diatoms such as *Nitzschia closterium* and *Navicula torquatum* are multiplied by photosynthesis The immense crops which are formed when seasonal influences are favourable provide the food for small zooplankton particularly Crustacea These animals absorb small amounts of unchanged β carotene from the diatoms but their main carotenoid is astaxanthin which is presumably formed from carotene As already mentioned vitamin A makes its appearance in shrimps Both blue and fin whales subsist on enormous masses of shrimps and build up their high reserves of vitamin A with only this animal as the main intermediary step from the diatom

References p 147

Not all small Crustacea have been shown to produce vitamin A, and there have been suggestions that astaxanthin and other substances as yet unidentified may serve as provitamins Thus Grangaud and Massonet [34] have claimed that astaxanthin has at least partial vitamin A activity Lane [35] reported that a substance with absorption at 310 mμ could be isolated from the (zooplankton) *Temora turbinata* and *Centropages typicus* When this substance was given to the fish *Limanda ferruginea* the concentration of vitamin A in its liver, as measured by the antimony trichloride test, was increased

After these obscurities during the crustacean stage of the ladder the further steps upwards seem relatively clear Small fish are eaten by larger fish, which are eaten by even larger fish or sharks Huge accumulations of vitamin thus occur in the livers of the tunny fish and in those of many species of sharks Seals eat fish and take over the large stores of vitamin into their own livers At the top of the ladder we find the polar bear, which preys upon seals with its liver containing up to 20,000 i u of vitamin A per g [36]

Marine detritus

Finally in the marine kingdom we may continue our story beyond the field of biology into the fringes of geology Thus in California it has been shown by Fox and his colleagues [37, 38, 39] that the mud on the sea bottom may contain substantial amounts of carotenoids Presumably the processes of fossilisation in the marine detritus favour the preservation of carotenoids by taking place at a low temperature and in absence of oxygen and light An interesting aspect of this phenomenon is the high ratio of carotene to other pigments in the mud, which greatly exceeds the ratio in the biological materials from which the detritus must have been derived

Conclusions

In this brief review we have seen that animals vary greatly in the choice of the carotenoids which they include in their blood and tissues and in the amounts of vitamin A which they accumulate in their livers

β Carotene runs through the whole natural order from many unicellular organisms right up to man Some animals, however convert virtually all the carotene which they absorb into preformed vitamin A Xanthophylls are preeminently the characteristic pigments of birds and of some of the lower orders including amphibia and coelenterates Man is rather unselective in his absorption of carotenoids and his body, in addition to carotene, may contain not only small amounts of xanthophylls but also lycopene The acidic astaxanthin is found most consistently in the Crustacea but among the lower forms of life it occurs in certain marine algae and in higher forms of life in the salmon and flamingo

The Crustacea, and possibly also the cephalopods, appear to be the lowest orders in which preformed vitamin A makes its appearance So far these ani-

mals if we discount traces of the vitamin in certain gastropods and bival (Chap 39) make the only exceptions to the generalisation that vitamin is characteristic of the vertebrates Although locusts resemble the Crusta in being arthropods and although they further resemble them in hav astaxanthin as their main pigment there is no evidence that vitamin A ever present in their bodies

At the top of the ladder at least in their vitamin A metabolism we fin selection of predatory animals including whales sharks seals the giant n nitor and the polar bear In these animals large reserves of vitamin A accumulated in the absence of more than small amounts of unchanged ca tenoids

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PART V

THE PHYSIOLOGY AND BIOCHEMISTRY OF VITAMIN A AND ITS PROVITAMINS AND CONGENERS

CHAPTER 14

General Introduction to the Absorption of Vitamin A and its Provitamins

There are many complicating factors, both chemical and physiological, which will make it difficult to give an account of the absorption of vitamin A, and its provitamins which is both clear and reasonably comprehensive.

From the start we must realise that the preformed vitamin, and its esters, are much more readily soluble in oils and fats than are the provitamins Probably this difference at least partially explains why provitamins are usually absorbed much less efficiently than the preformed vitamin We must next remember, however, that in many animals provitamins undergo conversion to vitamin A before passing through the intestinal wall The question of the absorption of provitamins, therefore, cannot be clearly separated from the question of their conversion In some circumstances the limiting factor in the ab

ad

agents which affect the degree of dispersal of dietary fat, and of pro- and anti-oxidants which affect the stability of its constituents These agents will vary considerably in their action on the vitamin and on its provitamins The absorption of vitamin A will depend upon whether it is given as the free alcohol or as its esters, and even different esters may vary in the efficiency of their absorption Each provitamin will differ in the efficiency of its absorption and utilisation, and the influence of *cis trans* isomerism must not be overlooked

Nc

or

relate findings which have been made by several different procedures Thus some workers have studied the "absorption" of vitamin A or its provitamins

in the blood or liver Even the growth rate after dosing may sometimes be

References p 156

taken as an indication of the amount of vitamin which has been absorbed. Each of these methods provides useful information provided that its limitations are recognised, but it is not always easy to compare the results of authors who define absorption in different ways.

The absorption and storage mechanism of vitamin A and its provitamins in outline.

Before we deal in detail in subsequent chapters with each phase of our complex problem it may help some readers to have a brief summary of the main features in the absorption, storage and mobilisation of vitamin A. In all animals moderate doses of vitamin A disappear completely during the passage of food through the intestinal tract When provitamins are eaten, however, a considerable fraction is passed into the faeces Vitamin A, and probably other colourless derivatives, are formed from the part of the provitamins which disappears in the intestines. In animals with colourless body fat, which present the simplest picture, the conversion to vitamin A takes place mainly in the intestinal wall.

Both vitamin A eaten as such and vitamin A newly derived from carotene are carried to the liver at least partly by the thoracic duct. Absorption into the liver tissues occurs rapidly, but this does not prevent a substantial increase in the level in the blood plasma after heavy dosing. In most animals a maximum level in the plasma is found 4—6 hours after dosing with preformed vitamin A, but after carotene the maximum is less pronounced and is reached more slowly. There is also some evidence that the increases produced by provitamins are more prolonged than those produced by preformed vitamin A.

Even if vitamin A is ingested in the form of its free alcohol the increase in the plasma after heavy dosing is found to be mainly due to esters of the vitamin If vitamin A esters are eaten they are broken down and the alcohol is re-esterified, sometimes with other acids In contrast to the temporary increase after dosing, however, the vitamin A responsible for the "resting level" is present almost entirely in the form of the free alcohol. The picture changes again in the liver, where esters greatly predominate.

Many features of the mechanisms underlying these movements, and changes in the chemical state, have still to be worked out in detail In broad outline, however, it seems reasonable to assume that preformed vitamin A is hydrolysed, if necessary, and re-esterified during passage through the intestinal wall. After a few hours in the blood stream most of it is absorbed, still mainly in esterified form, by the liver. A delicately controlled mechanism, in which the Kupffer cells are probably involved, then allows the vitamin to be [illegible]

tually cause the level of vitamin in the plasma to fall, but heavy doses may have remarkably little permanent effect.

The blood may be supposed to carry the vitamin to all the tissues of the body The retina, the kidneys, the suprarenals and the lungs may be mentioned as sites where the vitamin reaches the highest concentrations outside the liver. In blood the vitamin is located in the plasma, with only traces in the corpuscles.

In animals, whose fat is coloured yellow by provitamins, we have to face additional problems Thus we must compare the amounts of provitamins which are converted to the vitamin in the intestinal walls with those which pass through unchanged, and must consider the fate of unchanged pigment after it has passed beyond the established zone for its conversion The best known instances of the fat being coloured by provitamin, moreover, are seen in man and the bovine Since they are less available, or less convenient, than small animals for submitting to drastic experimental operations it is perhaps not surprising that our information on these points is still incomplete.

TABLE 9

MAIN FEATURES IN THE TRANSFER OF VITAMIN A AND CAROTENE FROM THE DIET TO THE BLOOD AND LIVER

Diet	*Intestinal walls*	*Blood (post absorptive)*	*Liver*	*Blood (resting level)*
Carotene →	→ (↓ or converted to vitamin A ↓)	→ Carotene protein complex ?		
Vitamin A alcohol →	Esterified (↑ from Hydrolysed) →	Vitamin A esters →	Vitamin A esters (mainly) ←	→ Vitamin A mainly alcohol (As protein complex ?)
Vitamin A esters →	Hydrolysed (in lumen ?)			

Early observations In 1935 a patient suffering from a condition which caused part of the contents of the throracic duct to be diverted to the pleural cavity gave Drummond, Bell and Palmer [1] a good opportunity to study the absorption of vitamin A and carotene Clear evidence was obtained that carotene is less efficiently absorbed than the preformed vitamin Thus it was found that a relatively small proportion of carotene, administered orally, could be accounted for by the pigment present in the chylous fluid In contrast the large amount of vitamin A recovered indicated almost complete absorption

References p 156

When vitamin A was administered as the free alcohol it was found in the lymph mainly in esterified form, which suggested that the linkage of the vitamin with fatty acids during passage through the intestinal walls might account for the efficiency of its absorption Both vitamin A and carotene appeared to be present in colloidal form, and to be closely associated with the highly dispersed fat

The importance of bile Drummond and McWalter [2] recognised the importance of bile for the absorption of carotene, and demonstrated that the provitamin could form a complex with desoxycholic acid

Further evidence was obtained by Greaves and Schmidt [3] in studies on rats which were made jaundiced by ligating and sectioning the common bile duct Before their operations the rats were kept on a diet deficient in vitamin A until cornified cells appeared constantly in their vaginal smears Oral doses of halibut-liver oil given 24 hours after operating were usually effective in changing the vaginal smear, but carotene was ineffective Similar results were obtained when the operation was varied by connecting the bile duct with the upper part of the descending colon In some attempts, carotene could be made effective by the simultaneous administration of sodium desoxycholate or sodium glycodesoxycholate

Parenteral administration At this point it may be appropriate to mention the administration of the vitamin by injection, which might at first sight appear to circumvent the need for bile As a general rule however, both vitamin A and its provitamins are less effective when given parenterally than when given orally Thus Greaves and Schmidt found that carotene was ineffective in their jaundiced rats even when it was placed on the inner side of the intestinal barrier by subcutaneous or intraperitoneal injection

The effect of species In Chapter 13 we have discussed the variations in vitamin A reserves characteristic of different animal species To some extent these variations can be explained by feeding habits Thus predatory animals will enjoy large intakes of preformed vitamin A In contrast herbivorous animals will obtain vitamin A, once they are past the suckling stage entirely by the conversion of carotene

Even in animals subsisting on similar diets, however, it seems clear that species may influence the extent of the reserves of vitamin A which are accumulated in the liver We have seen in the preceding chapter that sheep have larger reserves than bovines subsisting on the same diet, and that rabbits have much larger reserves than guinea pigs The cause of these divergences

Thus guinea pigs have very low reserves of vitamin A if they are kept on diets containing liberal amounts of carotene, but high reserves after the administration of massive doses of vitamin A At one time the suggestion was made that carniverous animals are deficient in their power to absorb and convert provitamins Thus Ahmad [4] considered that carotene was poorly absorbed and converted in cats No comprehensive study of this problem appears to have been undertaken Since the guinea pig is not carnivorous however, it is clear that causes for the inefficient conversion of carotene need not necessarily be confined to the feeding habits of the animal

Modification in diet and dosing methods Numerous investigations to be described in detail later, have been made on the effects of minor dietary changes as opposed to gross alteration of the feedi habits, on the absorption of vitamin A and its provitamins Thus the pr sence of fat in the diet has been reported to assist absorption Lecithin al tends to aid absorption Medicinal paraffin carries unchanged carotene in the faeces but has little effect on the absorption of preformed vitamin A

In some circumstances emulsifying agents such as the Tweens have bee found to increase greatly the speed and efficiency of absorption of preforme vitamin A This effect can be observed most readily in human volunteer rats can absorb the vitamin so efficiently from fatty solutions that litt margin remains for the emulsifying agent to effect any improvement

mals deprived of vitamin E for long periods Other antioxidants have al been studied

Sex The convincing evidence that sex affects the metabolism of vitam A, and its provitamins will be given at length in Chapter 35 For th present it may be mentioned that females tend to have higher reserves vitamin A in their livers than males, and higher levels of carotenoids in the

Disease In addition to these dietary and physiological factors we sha later have to discuss the effects of disease (Chapter 32) Obvious our thoughts may turn first to conditions such as coeliac disease sprue ar fibrocystic diseases of the pancreas in which poor absorption of fat is a pr minent feature A wide variety of other diseases, however, must also be con sidered Thus the metabolism of vitamin A is always affected in fever E tensive studies of these disturbances have been made in infective hepatit

References p 156

and rheumatic fever Evidence that the conversion of carotene to vitamin is sometimes defective has also to be reviewed with particular reference to thyroid abnormalities (Chapter 37) In some diseases particularly pneumonia and chronic nephritis substantial amounts of vitamin A but not provitamins are passed into the urine in making a balance sheet they must be deducted from the amounts absorbed from the diet

Finally in both man and animals the absorption of vitamin A and its provitamins may be adversely affected by infestation with intestinal parasites (see Chaps 32 and 34)

In succeeding chapters we must review our knowledge of the various aspects of the absorption of vitamin A and its provitamins in greater detail and discuss the evidence on which it is based

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CHAPTER 15

The Absorption of Provitamins

It was emphasised in the preceding chapter that the mechanisms for the absorptions of vitamin A and of it provitamins involve separate problems although obviously common factors are involved The present chapter is therefore confined to the absorption of provitamins except in so far as it is necessary to contrast their absorption with that of vitamin A Moreover in view of the wide field to be covered even after this limitation it will be convenient to subdivide the subject still further We shall first proceed therefore to describe the absorption of provitamins in general terms and to compare the different efficiencies of absorption of various provitamins and of the *cis trans* isomers of the same provitamin Discussion of the effect of dietary modifications on the absorption of provitamins will be postponed to the following chapter

State of carotene in foods The influence of the low solubility of carotenoids on their ease of absorption has already been mentioned In the laboratory it is difficult to make stable solutions of β carotene in arachis oil which is often used as a diluent containing more than about 1 mg per g (0 1%) About the same concentration is found in strongly coloured specimens of red palm oil [1] If stronger solutions are made by dissolving the carotene in benzene mixing it with the oil and completely removing the solvent by evaporation the pigment slowly crystallises during storage

The concentration of carotene in butter fat which is one of the most familiar sources of carotene in human nutrition is always well below the saturation level Some vegetable foods however contain much more carotene than their fat would be expected to dissolve Thus in carrots if we take 12 mg per 100 g as typical of the carotene content and 0 4 g as the fat content the concentration of carotene in the fat works out at 3% Thirty times more carotene is present therefore than could be dissolved in a corresponding amount of arachis oil This doubtless explains why carotene tends to crystallise spontaneously from fat extracted from carrots

It seems probable, however that the pigment is held in plant tissues in

References p 164

forms other than a simple solution in fat. Thus Strauss[2] has reported that the juice pressed from carrots contains a carotene-protein complex from which the pigment cannot be extracted by ether or light petroleum. Similar problems have to be faced with green vegetables, with the additional complication that xanthophyll in twofold excess over the carotene competes for about the same amount of fat. It cannot be excluded, of course, that the carotenoids are sometimes deposited in plant tissues in crystalline form.

The limited solubility of carotene in edible oils introduces difficulties when it is desired to give massive doses for experimental purposes. Large amounts of the pigment have sometimes been given in crystalline form, but the efficiency of absorption must have been very low.

The incomplete absorption of provitamins

As the result of extensive biological tests with rats it has been concluded that the international unit of β-carotene, 0.6 μg, is almost exactly equivalent to 0.3 μg of vitamin A.[3] This relationship, however, only applies when the two substances are compared at low levels of dosing, and when the basal diet and experimental conditions do not differ widely from those generally considered suitable for accurate biological tests.

To explain the inferiority of β-carotene to vitamin A we must assume either that half of each molecule of the pigment is wasted during conversion, or alternatively that out of every two molecules one is wasted and the other converted into two molecules of vitamin A. To help us in choosing between these two alternatives we know that β-carotene, with its two β-ionone rings, is at least twice as biologically effective as other provitamins, which have only one β-ionone ring. Obviously this superiority can be explained most simply on the assumption that two molecules of vitamin A are derived from each β carotene molecule which undergoes conversion. The greater activity of vitamin A appears to indicate, therefore, that even under conditions for absorption and conversion which approach the optimum only about 50% of the ingested carotene is used for making vitamin A. Presumably the remaining molecules are either not absorbed, or are metabolised or destroyed without conversion to the vitamin*.

When higher levels of carotene and vitamin A are given the disparity

* J. Glover and E. R. Redfearn[46] consider that the conversion of carotene may not take place by central fission, but by stepwise degradation from one end of the molecule. To explain the twofold biological superiority of β carotene over α carotene on this basis we must assume that every molecule of the β isomer which is degraded gives a single molecule of vitamin A. With α carotene vitamin A is formed when degradation starts at the end of the molecule remote from the β ionone ring, but an inactive product results when the β ionone ring is itself degraded. On the assumption that the attack on the molecule can occur equally easily at each end it is obvious that one molecule of vitamin A will be produced from every two molecules of α carotene that are attacked.

between the efficiency of their absorption as indicated by the storage of vitamin A in the liver is greatly increased An illustration of this point may be taken from early unpublished work by the author When rats were given a diet containing about 20 mg of carotene per 100 g or the same amount of a vitamin A concentrate the rate of storage of vitamin A in the liver, examined at periods up to 12 weeks was 5–10 times greater in the animals given con centrate than in those given carotene (see Table 10) Since the concentrate contained less than 50% of vitamin A the actual disparity was 10–20 fold

These differences it should be noted are observed when roughly equi valent doses of carotene and vitamin A are given By removing this condi tion and by comparing simply the maximum rates at which vitamin A may be absorbed into the liver from liberal sources of either provitamins or pre formed vitamin, the difference may be increased to 100 fold To quote again from the author s early work [4] it would appear that in rats given lavish amounts of carotene either in oily solution or as carrots the rate of storage of vitamin A does not exceed about 200 i u per day More recent experience has often shown that rats can readily absorb 20 000 i u in 24 hrs if a sufficiently massive dose of preformed vitamin A is given

A familiar instance of the poor absorption of carotene may be seen in the dairy cow when at pasture Only a minute fraction of the carotene available in the herbage is either absorbed unchanged or converted to vitamin A (see Chap 13)

TABLE 10

AN EARLY EXPERIMENT BY MOORE ON THE RELATIVE RATES OF ACCUMULATION OF VITAMIN A RESERVES BY YOUNG RATS GIVEN LARGE ALLOWANCES OF EITHER CAROTENE OR VITAMIN A CONCENTRATE

		Total vitamin A reserves in i u	
Days on experiment	*Initial reserves*	*Diet * with vitamin A*	*Diet with carotene*
0	75		
	45		
	70		
	50		
28		30 000	3 500
		40 000	4 000
70		60 000	15 000
		75 000	15 000
84		75 000	14 000
		90 000	15 000

* The diet contained casein 20% sugar 42% coconut oil 22% dried yeast 8% wheat germ 4% and minerals 4% The vitamin A or carotene was dissolved in the coconut oil and the diet mixed fresh daily

References p 164

The relative efficiencies of utilisation of α β and γ carotenes Little evidence is available about the relative efficiencies of absorption of different provitamins as measured by their disappearance in passage through the intestinal tract Comparisons based on growth promotion or the storage of vitamin A in the liver will obviously be influenced not only by absorption but also by the efficiency of conversion as determined by chemical structure It seems appropriate however to mention such comparisons at this point

Soon after the separation of α and β carotenes Kuhn and Brockmann [5] found that β carotene was about twice as effective as α carotene in promoting growth and the storage of vitamin A in rats Examination of pigment in the faeces or of the small amounts which reached the tissues without conversion to vitamin gave no evidence of interconversion between the α and β isomers γ Carotene had the same activity as α carotene [6] Later Wilkinson [7] made careful biological tests on carotenes isolated from red palm oil The activity of the β isomer was 2 0 × 10[6] i u per g and that of the α isomer 0 92 × 10[6] i u To explain an activity greater than the theoretical maximum of 1 67 × 10[6] i u per g for the β isomer it was suggested that the specimen used for the international standard was only 90% pure In experiments on the accumulation of liver reserves Johnson and Baumann [8] found a greater disparity between α and β carotenes the reserves produced from the α isomer being only 25% of those produced from the β isomer

Cryptoxanthin This provitamin which is important in diets containing substantial amounts of maize, was found by Kuhn and Grundmann [9] to have between one quarter and half the potency of β carotene Later a more accurate investigation by Deuel and his colleagues [10] indicated 54–59% of the activity of β carotene With [11] deduced from indirect evidence that the activity of cryptoxanthin in hens exceeded that of carotene and about equalled that of vitamin A He suggested therefore that cryptoxanthin and other substances capable of conversion to vitamin A might in some circumstances exert their physiological action without undergoing conversion Patel Mehl and Deuel [12] however proved that in the rat cryptoxanthin is converted to vitamin A in the intestinal walls

Johnson and Baumann [13] found that although cryptoxanthin had only about half the growth promoting activity of β carotene the two substances were about equally effective in causing the storage of vitamin A in the liver

Cis trans *isomerism* Deuel Zechmeister and their colleagues published an extensive series of papers on the effect of *cis trans* isomerisation on the biological activity of carotenoids [14–23] In general *cis* compounds which seem often described by the prefix *neo* if they are obtained artificially or *pro* if they occur naturally have potencies much inferior to

those of the corresponding all *trans* compounds Table 11 gives the relative potencies taken or calculated from Deuel s communications It will be seen that pro γ carotene is exceptional in being at least as potent as the all *trans* isomer At first it was found that pro γ carotene isolated from ripe *Pyracantha* berries had 44% of the activity of β carotene as compared with only 28% found for the all *trans* modification isolated from mimosa blossoms Later the all *trans* γ carotene as well as pro γ carotene appears to have been isolated from *Pyracantha* berries The activity of the all *trans* form was re estimated as 45% of that of β carotene which was virtually the same as 44% which had been previously found for pro γ carotene

TABLE 11

THE RELATIVE POTENCIES OF *cis trans* ISOMERS

	Potency
All *trans* β carotene	100
Neo β carotene B	53
Synthetic 15 15 mono *cis* β carotene	50
Neo β-carotene	38
All *trans* α carotene	53
Neo α carotene B	16
Neo α carotene U	13
All *trans* γ carotene	28 or 45
Pro γ-carotene (poly *cis*)	44
Neo-γ carotene P	19
Mixed neo γ carotenes	16
All *trans* cryptoxanthin	57
Neo cryptoxanthin	42
Neo cryptoxanthin U	27

Homo β-carotene Lengthening of the central chain in the carotene molecule also causes a reduction in biological activity Thus Deuel Inhoffen Ganguly Wallcave and Zechmeister [24] found that synthetic all *trans* homo β carotene $C_{42}H_{58}$ had only 20% of the potency of β carotene The di *cis* 16–16 isomer had the same activity

The excretion of provitamins in the faeces Early studies by Moore [25] Ahmad [26] Wilson Ahmad and Majumdar [27] and De [28] demonstrated that rats excreted large amounts of their dietary carotene in their faeces The proportion excreted which depended on factors which will be mentioned in our next Chapter sometimes reached 90% Ramasarma and Hakim [29] made the interesting observation that rats secrete traces of a yellow pigment equivalent in colour to 0 2–0 4 μg of carotene daily even when they are restricted to a diet deficient in vitamin A

References p 164

This pigment, which could be separated from β carotene on a chromatogram had no biological activity when tested in doses which were colorimetrically equivalent to effective doses of β carotene In studying the absorption of very small doses of carotene the presence of this pigment had obviously to be taken into account

Johnson and Baumann[13] gave doses of 2–78 μg of β carotene, *a carotene* and cryptoxanthin to rats The percentages recovered from the faeces were 40, 54 and 36 respectively, the same values being found repeatedly for each pigment irrespective of the size of the dose

Studies of the absorption of carotenoids in man have been made by many workers[30-36] As in the rat a large fraction of the pigment present in the diet is excreted in the faeces In a careful study undertaken in Britain for the Medical Research Council average excretions of between 26 and 75% were found according to the source of carotene which was eaten[37]

The administration of carotene by injection In 1930 Rydbom[38] restored growth in rats deficient in vitamin A by injecting an oily solution of carotene into their leg muscles Carotene could be recovered unchanged from the injection sites, but only a faint antimony trichloride re action was given by fat extracted from the liver Later Wilson Ahmad and Majumdar[27] confirmed that carotene was effective when given by injection In rats repeated intraperitoneal injections of carotene as a colloidal suspen sion in isotonic glucose, both restored growth and cured xerophthalmia The presence of unchanged carotene at the injection sites was confirmed but it was claimed that considerable stores of vitamin A were deposited in the liver In rabbits the colloidal solution of carotene was injected by the ear vein It is not clear whether the injections were effective in curing deficiency, but interesting observations were made in the fate of the pigment In confirmation of an early report by Van den Bergh, Muller and Broekmeyer[39], which had been confirmed by Drummond and McWalter[40], carotene was taken up by the liver and a smaller amount was found in the lungs Even large quantities, however appeared in the faeces, particularly when a long interval was allowed between the injection, or series of injections, and the sacrifice of the animal The colloidal solution of carotene was presumably prepared by the method of Foder and Schoenfeld[41] A solution of the pigment in acetone is poured into cold water and the acetone is removed by evaporation under reduced pressure

Further experiments on rats were made in 1942 by Lease Lease Steenbock and Baumann[42] They confirmed the ability of carotene to restore growth and cure xerophthalmia in deficient animals when it was injected intraperitoneally or subcutaneously as an oily solution or as an aqueous col loidal suspension The doses required, however, were 10–100 times greater

than those needed when given orally Not even a total dose of 7 5 mg of carotene spread over 5 weeks caused the appearance of vitamin A in the liver. The inefficient utilisation of the carotene was confirmed, moreover, by the detection of particles of pigment at the sites of injection, and also in the liver, omentum and lymph nodes in the peritoneal cavity. Much of the carotene in these sites appeared to be immobilised, since the particles were sometimes present in animals which had died with symptoms of deficiency. Much of the injected carotene could not be traced, the loss could be explained neither by its conversion to vitamin A nor its excretion in the faeces

The remarkable effect of a modern detergent on the utilisation of injected carotene was studied by Tomarelli, Charney and Bernhart [43] Groups of rats deficient in vitamin A were given single doses of 0 44 mg of carotene either orally or by injection, and made up either in oily solution or as a colloidal solution stabilised by Tween 80 which is a polyoxyethylene derivate of sorbitan monooleate The average responses were as follows

Medium	*Method of dosing*	*Average maximum weight attained g*	*Average time of survival days*
Tween	Injection	140	71
Tween	Oral	125	40
Oil	Oral	115	40
Oil	Injection	90	20

It will be seen that Tween so much facilitated the absorption of the injected carotene as to make the pigment more effective by this route than by mouth. On the other hand the poor utilisation of carotene injected in oily solution was confirmed In agreement with these findings carotene which had been administered in Tween was found to disappear from its injection sites much more rapidly than carotene which had been administered in oily solution

Sexton, Mehl and Deuel [44] were struck by the contrast that whereas oral doses of carotene caused the appearance of vitamin A in the livers of their rats parenteral doses caused the appearance of unchanged carotene It was confirmed that the rat could die from vitamin A deficiency while its liver still contained large amounts of carotene These observations will require further comment when the question of the conversion of carotene to vitamin A in the intestinal wall comes under discussion (Chap 17)

From experiments with dogs Kowalewski, Henrotin and Van Geertruyden [45] concluded that carotene in oily solution is absorbed by the thoracic duct, but that emulsified carotene is absorbed by the portal vein.

References p 164

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CHAPTER 16

Dietary Factors which Influence the Absorption of Provitamins

Extensive studies have been made on the effect of the other ingredients of the diet on the absorption of provitamins Since the carotenoids are absorbed in association with fats the amount of fat in the diet had obviously to be considered As another form of the same problem, moreover, the absorption of provitamins present in vegetable tissues had to be compared with that of the same provitamins when supplied in oily solution The effect of emulsifying agents and stabilisers had to be investigated, in the anticipation that they would help in the absorption of provitamins Conversely substances promoting oxidation, or carrying provitamins into the faeces, would be expected to affect their absorption adversely

Vegetable sources versus oily solutions of provitamins

Studies of the faecal excretion of carotenoids cannot always be relied upon to give an accurate indication of their absorption In spite of difficulties, however, this method has been used in numerous comparisons of the absorption of provitamins from various vegetable sources and from oily solution According to an early report by Wilson, Ahmad and Majumdar [1] rats excreted only 30% of their dietary carotene when it was given in the form of mango pulp, as compared with 45% when the carotene was given as a solution in olive oil Most workers have found, on the other hand, that provitamins are better absorbed from oily solution than from vegetables Thus experiments by De [2] indicated that about 45% of carotene was usually absorbed by rats from the green vegetable amaranth as compared with 65% from red palm oil The superiority of the absorption from the oil was most noticeable at high levels of dosing Kemmerer and Fraps [3] stated that when rats were given about 10 mg of carotene per kg of their diet they absorbed more than twice as much when the pigment was given dissolved in Wesson oil than when it was given as dehydrated alfalfa leaf meal They confirmed that the efficiency of absorption varied with the dose, and found that it also varied between the rat and chicken

Later the same workers [4] compared the absorption of carotene from vega-

tables and oily solution by measuring growth responses rather than by analysis of the faeces They concluded that when only small doses were given the absorption from all vegetables except carrots was as good as from oily solution

			Percentages of absorption with doses[3] of carotene mg/kg diet		
		Dose	1	10 5	20 mg
Rat	Oily solution		—	51	—
	Dried alfalfa		43	22	18–23
Chicken	Dried alfalfa		69	—	29

It must be realised that measurement of the absorption of carotene by analysis of the faeces is made difficult and uncertain by technical complications The state of the pigment in the dietary source and in the faeces will be widely different and there may be corresponding variations in the efficiency of extraction If the carotene is hard to extract from the dietary source but easier to extract from the faeces the pigment may appear not to be absorbed at all or even to be manufactured in the intestines These difficulties were at least fully realised in studies made during the Medical Research Council s experiments on vitamin A deficiency in human volunteers[5] The following average values were found for the carotene absorbed as measured by the difference between the contents of the food and faeces in numerous tests

	% absorbed
β Carotene in arachis oil	74
β Carotene in margarine	71
Spinach canned purée	41
Spinach canned homogenised	43
Cabbage dried outer leaves	41 or 27
Carrots canned homogenised	56
Carrots canned sliced	24
Carrots canned sliced purée	25

These results support the view that carotene is generally better absorbed from oily solution than from vegetables The comparison between the oily solution and carrots which had not been finely homogenised indicated three times better absorption from the oily solution Probably further research would show that the efficiency of absorption of provitamins from vegetables depends largely on the extent to which the vegetable tissues are comminuted

References p 178

and digested Examination of the faeces after a meal of carrots will often reveal the presence of large undigested fragments of the vegetable

As will be seen later still further complications in assessing the efficiency of absorption of provitamins are introduced by factors which affect their stability during passage through the intestines No satisfactory method for calculating the quantitative effects of these factors has been worked out, and estimates of the efficiency of absorption by measurements of faecal excretion at the best give only rough approximations

The quantity of fat in the diet In 1929 Moore [6] found that the removal of the usual fat component from the basal diet of rats did not noticeably affect the biological activity of a liberal dose of carotene, which was administered in a drop of arachis oil In rats given various sources of carotene Wilson, Ahmad and Majumdar [1] confirmed that the inclusion or exclusion of fat had little effect on growth, but the reserves of vitamin A in the liver tended to be higher in those groups which had been given fat than in those which had not Majumdar [7] went a stage further by demonstrating that rats could be cured of xerophthalmia by aqueous colloidal solutions of carotene even when fat had been removed from their diet by extraction with ether Randoin, Hugot and Causeret [8] varied the usual procedure by mixing the carotene with the diet, rather than by giving it as a separate dose, but again found that the growth responses obtained were unaffected by the presence or absence of dietary fat

According to other investigators, however, the quantity of dietary fat seems more important Thus Russell, Wight Taylor, Walker and Polskin [9] reported that hens absorbed less carotene when their diet contained only 0 1% of fat than when 4 0% was included Later work on rats by Burns, Hauge and Quackenbush [10] indicated that carotene caused better growth responses when the diet contained 5% of lard than when the animals received no fat, beyond their daily requirement of unsaturated acids in the form of maize oil

The quality of the fat The nature of the fat used either as a dietary component or as a diluent for carotene may affect the absorption of the provitamin in various ways It will be recalled that early research on the biological activity of carotene was hindered by its instability when dissolved in various fatty media Ability to preserve the pigment, which depends partly on the presence of the tocopherols and other natural anti oxidants, is perhaps the most important qualification of a fat for aiding the absorption of provitamins The digestibility of the lipoid medium, however, must not be ignored Thus the inclusion of medicinal paraffin in the diet [illegible] carotene Other poorly absorbed

In studies on the conditions necessary for the use of carotene as an international standard for vitamin A Dyer Key and Coward [11] found that the provitamin was 5–6 times more potent for rats when dissolved in arachis oil than when dissolved in hardened cotton seed oil or ethyl laurate Solutions made up in arachis oil and coconut oil were usually equally effective but one out of three samples of coconut oil gave solutions of low activity Lathbury and Greenwood [12] confirmed the variations between different samples of coconut oil and also found similar variations in arachis oils No simple relationship could be found between the state of oxidation of an oil and its unsuitability as a diluent for carotene Attempts to improve oils which were poor diluents for carotene by the addition of hydroquinone were not always successful

Kraybill and Shrewsbury [13] compared the growth promoting powers in rats of graded doses of carotene when given either in cotton seed oil or in butter fat which had been decolourised by Lloyd s reagent The provitamin was 2–3 times more effective with the cotton seed oil as diluent than with the butter fat Lease Lease Steenbock and Baumann [14] however found that carotene was equally well utilised by rats either for growth or for the storage of vitamin A from cotton seed oil decolourised butter fat soya bean oil lard coconut oil and crude peanut oil In their experience inferior responses were obtained with triolein linseed oil and refined peanut oil Sherman [15] obtained the best growth responses in rats with soya bean oil as diluent the next best with cotton seed oil linseed oil maize oil and wheat germ oil and the worst with butter fat and coconut oil Methyl linolate inhibited the action of small doses of carotene unless an interval of several hours was allowed between the doses of the two substances As Sherman [16] pointed out his findings on the different natural oils could largely be explained by a physiological antagonism between unsaturated fatty acids and vitamin E in influencing the metabolism of carotene This idea was in keeping with a previous observation by Moore Martin and Rajagopal [17] that deficiency of vitamin E in rats caused defective storage of vitamin A

At this period therefore the role of antioxidants tended to monopolise attention in investigations on the influence of the quality of the dietary fat on the metabolism of provitamins An interesting paper by Brown and Bloor [18] however stressed the importance of other factors Rats were given diets which included 10% of various fractions from the fatty acids of butter and carotene was supplied in the form of 3 g of raw carrot weekly Comparisons were made of the efficiency of digestibility of the fractions and of the concentration of vitamin A accumulated in the liver It will be seen from Table 12 that greater stores were accumulated by the rats given the well absorbed non volatile liquid fractions than by those given the poorly ab

References p 178

sorbed solid fractions The low storage of vitamin A in the animals given the volatile fraction was not fully explained but possibly this fraction was injurious

TABLE 12

THE DIGESTIBILITY BY RATS OF VARIOUS FRACTIONS OF THE FATTY ACIDS OF BUTTER AND THEIR INFLUENCE ON THE STORAGE OF VITAMIN A DERIVED FROM CAROTENE FROM DATA BY BROWN AND BLOOR (1945)

Fraction	*Percentage absorption*	*Vitamin A i u /g liver*
Volatile	90 6	632
Low M W liquid	95 0	1870
High M W liquid	93 3	2070
Low M W solid	71 3	1150
High M W solid	42 2	1320

The influence of tocopherol and other antioxidants

In the first detailed account of the effect of vitamin E on the storage of vitamin A Moore [19] reported that vitamin E had much less effect on the storage of vitamin A derived from carotene than on the storage of preformed vitamin A This difference was attributed at the time to variations in the experimental procedure during different stages of the investigation Soon afterwards the problem was approached from a different angle by Hickman and his colleagues [20 21 22] who studied the effect of tocopherol in increasing the growth responses in rats given small doses of carotene Daily doses of 0 5 mg of mixed tocopherols increased the responses caused by 1 μg of carotene but it was noticed that with larger doses of tocopherol the effect was reversed The effect could only be demonstrated moreover when the doses of carotene were marginal Growth responses with high doses of carotene were little effected by the tocopherol level

Evidence was obtained by Hickman and his colleagues that the tocopherol acted by reason of its antioxidant powers on stabilising carotene in the intestinal tract Thus free tocopherols were much more effective than esterified tocopherols which fits in with the finding that the tocopherols only protect fats from oxidation *in vitro* when they are added in the free form Similar effects on growth responses were given by lauryl hydroquinone ascorbic acid and palmityl ascorbic acid but not by hydroquinone or *p* aminobenzoic acid Tests on human volunteers suggested that tocopherol increased the excretion of carotene in the faeces As already hinted it became apparent that tests on the absorption of carotene by measuring the faecal excretion are open to criticism By dosing with tocopherol it was possible both to increase the actual absorption as measured by growth rate in rats

and at the same time to diminish the apparent absorption, as measured by the amount of carotene found in the faeces.

Quackenbush, Cox and Steenbock [23] followed the lead given by Sherman, and examined the effect of α-tocopherol, and other antioxidants, in increasing the stability and growth-promoting power of carotene dissolved in ethyl linolate. For increasing the activity of daily doses of 5 μg of carotene concentrations of 0 01–0 03% of α-tocopherol in the dosing solutions were found to be necessary, which were equivalent to daily doses of 2–6 μg Vitamin K, pyrogallol, catechol and hydroquinone were inactive at 0 02%, but hydroquinone was active at 1% Both α-tocopherol and hydroquinone were equally effective as antioxidants *in vitro* but hydroquinone, unlike tocopherol, could be removed from the oily solution by extraction with water The low activity of hydroquinone in animal tests therefore appeared to be due to its transfer from the oily to the aqueous phase in the intestinal tract

Guggenheim [24] studied the efficiency of absorption of carotene from various vegetables as measured by the accumulation stores of vitamin A in the livers of rats He concluded that the efficiency of storage depended upon the tocopherol content of the vegetable In experiments in which a fixed dose of pure carotene was given, in conjunction with graded doses of α-tocopherol, clear evidence was obtained that the tocopherol simultaneously increased both the efficiency of utilisation of the carotene and also the amount excreted in the faeces (see Table 13) Tomarelli and Gyorgy [25] made the interesting observation that rice bran extracts contained a factor which could further support

TABLE 13

EFFECT OF α-TOCOPHEROL ON THE AVERAGE STORAGE OF VITAMIN A IN THE LIVER, AND EXCRETION OF CAROTENE IN THE FAECES IN GROUPS OF 8 RATS GIVEN 0 08 mg OF CAROTENE ON TWO SUCCESSIVE DAYS (GUGGENHEIM, 1944)

Daily dose of α tocopherol mg	*Total vitamin A in liver (i u)*	*Total excretion of carotene, μg*	*% of intake excreted*
0	4 5	30	19
0 1	6 5	38	24
0 5	11 0	47	29
2 0	15 0	55	36
10 0	18 0	66	41

the action of tocopherol in increasing the growth responses with small doses of carotene. A similar synergism was found in experiments *in vitro* on the protection of carotene Rao [26] found that the difference between the growth responses produced by solutions of carotene in arachis oil, coconut oil and

References p 178

olive oil were greatly reduced when the vitamin E contents of the oils were equalised by adding tocopherol to the coconut and olive oils

Against this run of evidence in favour of tocopherol increasing the efficiency of utilisation of carotene Johnson and Baumann [27] drew attention to an important qualification to the general rule We may remember that Hickman had noticed that whereas small doses of tocopherol increased the utilisation of carotene as measured by growth responses large doses had the reverse effect The Madison workers found under their experimental conditions that small doses of tocopherol had little effect on the utilisation of carotene as measured by the storage of vitamin A On the other hand large doses considerably decreased the storage of vitamin A Thus when rats were given daily doses of 40 μg of β carotene in conjunction with 0 0 5 2 5 5 or 1 0 mg of α tocopherol the total amounts of vitamin A stored in their livers were 30 5 31 1 29 8 17 and 6 6 μg Perhaps rather unexpectedly 5 mg doses of tocopherol given by injection also depressed the storage of vitamin A but oral doses given at an interval of 8 hours after the carotene doses had little effect In further work Swick and Baumann [28] found that high doses of α tocopheryl acetate and γ tocopherol had the same effect as α tocopherol Diamylhydroquinone also slightly decreased the storage of vitamin A

This additional twist to a story which may already have seemed somewhat complicated may perhaps make readers suspicious that all the results obtainded so laboriously on the absorption of carotene should be treated with scepticism It seems probable however that we must be prepared to coordinate a series of true pictures which reveal a complex set of interrelationships Thus in regard to the effect of vitamin E the results of different investigations are probably influenced by the severity of the deficiency of this vitamin to which the animals are exposed The same findings can hardly be expected if in some experiments tocopherol is given to correct a severe deficiency but in others only to reinforce supplies which are already available in the diet The effect of heavy doses of tocopherol in depressing the storage of vitamin A after carotene feeding may possibly result from inhibition of the oxidation of carotene to retinene (vitamin A aldehyde) which is probably a step in the conversion of the provitamin Thus small doses of tocopherol may favour the formation of vitamin A by preventing the complete and destructive oxidation of carotene whereas large doses may depress vitamin A formation by preventing even the first reversible oxidation to retinene

According to High Woods and Wilson [29] other antioxidants may also vary in their effect on carotene metabolism according to dosage Thus in rats daily doses of 0 5 mg of acetylhydroquinone increased the stores of vitamin A accumulated after 50 i u doses of carotene but 10 mg doses of the antioxidant caused decreased storage

Medicinal paraffin In 1927 Burrows and Farr[30] and also Dutcher, Ely and Honeywell[31] reported that rats were unable to use the vitamin A of butter fat when it was mixed with liquid paraffin Moness and Christianson[32] were unable to observe the same effect when cod liver oil was mixed with paraffin, and Rowntree[33] concluded that paraffin only suppressed the action of vitamin A when the diet contained small amounts of the vitamin Jackson[34] found that the antagonism between butter fat and paraffin was minimised when they were given at different times of the day

In experiments leading up to the differentiation between carotene and preformed vitamin A Moore[35] had noticed that carotene was less effective in promoting growth in rats when it was dissolved in paraffin than when it was dissolved in arachis oil Dutcher Harris Hartzler and Guerrant[36] followed up this clue, and proved that paraffin had a much greater effect on the absorption of carotene than on that of vitamin A Thus paraffin had no effect on the growth responses caused by cod liver oil but could completely prevent the effect of small doses of carotene The addition of hydroquinone to the paraffin solution of carotene had no effect in increasing its activity which indicated that the loss of activity was not due to destruction by oxidation It was established however that much larger amounts of carotene were excreted in the faeces when the carotene was dissolved in paraffin than when it was dissolved in ethyl laurate It was therefore concluded that the hydrocarbon carotene was more soluble in the unassimilated hydrocarbons of the mineral oil than in the lipids of the digestive juices which caused it to be carried through with the faeces Preformed vitamin A, on the other hand was presumably more soluble in the digestive juices than in paraffin

With[37] confirmed the effect of paraffin in depressing the absorption of carotene in rats given various sources including carrot meal Curtis and Kline[38] studied the importance of the phenomenon in human nutrition When volunteers on a constant diet were dosed with carotene dissolved in arachis oil the level of yellow pigments in their blood increased but no increase was observed if paraffin was added to the arachis oil Similarly when volunteers were changed to a diet which was rich in natural sources of carotene there was a large increase in the blood carotenoids but no increase was observed if small doses of carotene were given before each main meal The administration of a single large dose of paraffin at bed time did not reduce the blood carotenoids Alexander *et al*[39] carried out similar experiments without giving the volunteers extra carotene In subjects receiving ordinary diets the administration of various doses of paraffin either without emulsion or in the form of a commercial salad oil, caused considerable decreases in the blood carotenoids, often to only 50% of their original level

The depression of the absorption of carotene seems a valid argument

References p 178

ago not too f- . objection has follow- escape absorption, small amounts may be detected in the liver and other organs It is not surprising, therefore, that public health authorities should disapprove of the inclusion of paraffin in foods such as cakes and salad creams

Lecithin Evidence that the utilisation of carotene is aided by lecithin, or some substance closely associated with lecithin, was reported in 1943 by Slanetz and Scharf [40] Rats which were given a diet which contained purified B vitamins and choline in place of the usual dried yeast, and with cotton seed oil as a source of vitamin E, failed to respond normally to daily doses of 2 i u of carotene Normal growth responses to the carotene were observed, however, after the addition of 1% of soya bean phosphatides to the diet Similar claims for the influence of phosphates on vitamin A metabolism and also on vitamin E metabolism, were made by Polskin [41] and by Patrick and Morgan [42] as the result of work with chicks

Jensen, Hickman and Harris [43] could not confirm that phosphatides had any influence on vitamin A metabolism They pointed out that impure soya bean phosphatides often contained considerable amounts of vitamin E, to which the effects observed by the other workers could be ascribed Scharf and Slanetz [44] replied by comparing the effect of the additions of soya bean phosphatides or of α tocopherol to their basal diet The growth responses caused by doses of 10 i u of carotene were the same in rats given either the basal diet alone or the basal diet supplemented by tocopherol, but much greater responses were observed in animals given phosphatides It was suggested that the failure of Jensen *et al* to confirm the influence of phosphatides was due to the fact that their basal diet already contained phosphatides in the form of dried yeast

Later Slanetz and Scharf [45] found that the addition of soya bean lecithin to their basal diet greatly i
the livers of rats given 50
lecithin had been treated
lecithin increased the growth rate in rats given 33 i u doses of carotene daily The storage of vitamin A was only slightly increased Choline slightly increased the growth responses but did not affect the storage of vitamin A

Further research to decide whether the effects of lecithin are due to pure phosphatides, or to some impurity, seem highly desirable

Xanthophyll, other pigments, squalene, etc The carotenes, which act as provitamins in the animal body, present a strange contrast with the xanthophylls, which seem to have no essential role, except possibly in vision From time to time, however, workers have played with the idea that xanthophylls may have some obscure role in vitamin A metabolism

Evidence in favour of this view was obtained by Sherman[47] Rats receiving a diet deficient in both vitamins A and E grew more rapidly when they were dosed with 2 μg of carotene together with 5 μg of xanthophyll than when they were given only the carotene Since xanthophyll has no vitamin A activity itself it presumably acted by shielding the carotene possibly by diverting some of the lipoxidase activity of the alimentary tract which would otherwise have destroyed the provitamin Xanthophyll was less effective than tocopherol however in increasing the growth responses caused by carotene Templeton and Dudley[48] also concluded from experiments on chickens that xanthophylls helped in some way in vitamin A metabolism

Kemmerer Fraps and De Mottier[49] approached the problem from a different angle and obtained different results In seeking an explanation of the lower utilisation of carotene from vegetables than from oily solution they envisaged an interference by xanthophyll along the lines followed by certain antivitamins of the B group Thus xanthophylls might divert the enzyme systems necessary for the metabolism of carotenes by virtue of the structural similarity between the two classes of pigment In support of this theory it was found that the stores of vitamin A accumulated by rats dosed with carotene were decreased by about 20 per cent when xanthophylls were given as well Chlorophyll whose phytol portion is structurally related to the carotenoids had the same effect In neither case however was the difference in vitamin A storage as great as that found between rats given carotene either in oily solution or in vegetables Vavich and Kemmerer[50] followed with experiments on chicks When daily doses of about 100 i u of carotene were given the storage of vitamin A was not affected by daily doses of 600 μg of xanthophylls After raising the dose of carotene to 200 i u, however even doses of 100 μg of xanthophylls caused decreased storage of vitamin A

Callison *et al*[51] found no differences in the growth responses of rats dosed with carotene when graded dosed of lutein were given in addition They agreed therefore that the defective absorption of carotene from green vegetables as compared with oily solutions could not be explained by the presence of xanthophylls High and Day[52] stressed the importance of the size of the dose of lutein in deciding its effect on carotene metabolism Thus the amounts of vitamin A stored in the liver were increased by small doses of lutein but decreased by high doses The opposite effect of lutein at different doses was therefore similar to that found previously for tocopherol

High and Day also found that large amounts of squalene and phytol decreased the utilisation of carotene but smaller doses of α ionone β ionone and hydroquinone had no effect Later lycopene was found to improve the utilisation of carotene when it was given in small doses but large doses had

References p 178

no effect[53] In different experiments 2–6% of the ingested lycopene was recovered from the liver

Protein The possible effects of the protein allowance on the utilisation of provitamins have attracted little attention Fraps[54] found that changing the proportion of casein in the diet of rats from 18 to 36 % had little effect on either the absorption of carotene or on the storage of vitamin A James and ElGindi[55] compared the utilisation of carotene in rats which were first depleted of vitamin A and then, at the same time, were dosed with 35 i u of carotene daily and given modified diets containing different forms of protein Observations were made on the rate of growth, the faecal excretion of carotene, the storage of vitamin A in the liver and the level of vitamin A in the blood From Table 14 it will be seen that growth and the storage of vitamin A were both much lower in the groups given zein than in those given the other sources of protein At the same time the excretion of carotene in the faeces was lowest in the group given zein, but we have already seen that the proportion of carotene excreted is not always a reliable indication of the efficiency of absorption These results at least indicate that the influence of the dietary protein on carotene metabolism is worth further investigation

TABLE 14

THE EFFECT OF THE MAIN PROTEIN CONSTITUENT ON THE UTILISATION OF CAROTENE (AFTER JAMES AND ELGINDI 1953)

Averages for groups	*Main protein constituent of the diet*			
	Casein	*Lactalbumin*	*Gluten*	*Zein*
Food intake g rat 6 weeks	423	410	401	278
Final body wt g	207	198	170	126
Carotene in faeces μg in 6 weeks	191	282	255	162
Vitamin A total, i u, liver	1690	990	940	230

Coconut meal, effect of bulk According to Wilkinson and his colleagues[56, 57] the utilisation of carotene by rats was considerably influenced by the inclusion of coconut meal in their basal diet Growth was increased, whereas both the excretion of carotene in the faeces and the storage of vitamin A in the liver were decreased The meal [illegible] of carotene but
appe [illegible]
also [illegible]
by [illegible]
than improved, by coconut meal The excretion of carotene and the storage of vitamin A were unaffected It must be concluded therefore, that if

coconut meal is capable of modifying the metabolism of carotene at all the effect can be observed by modifying some basal diets but not others

Fraps [54] found that when cotton seed hulls were included as 50% of the basal diet of rats in place of starch, the bulk of the faeces was increased 10-fold The percentage of the dietary carotene excreted in the faeces was increased from 20 to 60 but the amount of vitamin A stored in the liver was unaffected The inclusion of agar as 50% of the basal diet also caused bulky faeces but the faecal excretion of carotene was not affected

Raw soya beans Soya beans contain an enzyme capable of oxidising fats which in turn oxidise carotene (see Chap 12) It is understandable therefore that the inclusion of raw soya beans in the diet should interfere with the utilisation of carotene Squibb Cannon and Allen [59] found that when cows were given carrot as a source of carotene in conjunction with a diet containing ordinary concentrated foodstuffs the level of pigment in the plasma increased When the concentrates included raw soya beans however, no increase in plasma carotene was observed

A brief summary of the diverse information included in this chapter is given in Table 15

TABLE 15

DIETARY FACTORS WHICH INFLUENCE THE ABSORPTION OR ULTILISATION OF PROVITAMINS

Increased utilisation	*Decreased utilisation*
Provitamins dissolved in oil	Provitamins supplied as indigestible vegetables
Fat included in diet	No fat in diet
Dietary fat readily digested	Fat or other lipoid poorly digested (e g high melting fat or medicinal paraffin)
Fat gives stable solution of provitamins	Fat gives unstable solutions
Antioxidants particularly tocopherols present in moderate concentrations	Pro oxidants such as methyl linolate included in diet Antioxidants in excessive doses
Small doses of lutein	Large doses of lutein squalene or phytol
Lecithin from soya beans	Raw soya bean meal containing lipoxidase
Protein in diet adequate	Protein in diet defective (zein)

References p 178

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CHAPTER 17

The Conversion of Provitamins to Vitamin A

The conversion of β-carotene to vitamin A, which requires the reduction of the molecule to half its original size and the addition of water, may seem a very simple process. Even 25 years after the discovery of the conversion, however, the mechanism underlying the change has not been worked out in full detail. Thus we have good grounds to believe that the pigment is first

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capable of oxidising carotenoids? Are the plant enzymes incapable of attacking carotene in the centre of its molecule? Or may the plants lack the reducing or stabilising systems which are necessary for the formation and preservation of vitamin A in the tissues?

Other problems remain to be faced concerning the site of the conversion For various reasons it was thought at first that the conversion was effected in the liver, and this view went unchallenged for many years A phase of rapid development then followed in which the application of modern chromatographic methods demonstrated clearly, in many animals, that conversion takes place in the intestinal walls For animals with colourless blood plasma and body fa

fidence is h

the bovine,

gested that various parts of the body, and particularly the liver, may be capable of converting any carotene which escapes conversion in the intestines.

Early evidence. To obtain proof of the formation of vitamin A from carotene Moore [1] first gave rats a diet deficient in vitamin A for several weeks. Some of the animals were then killed, and their colourless liver extracts were found to give no blue colour when treated with the antimony trichloride reagent. Large doses of carotene were then given for 2 or 3 weeks. The numbers of yellow and blue "Lovibond Units" in the carotene were calculated, and subsidiary feeding tests were undertaken to prove that the pig-

ment did not owe its biological activity to the presence of vitamin A as an impurity. This was done by comparing the minimal effective doses of carotene and vitamin A, and by showing that the blue colour of carotene was not sufficiently intense to mask the presence of enough vitamin A to explain its activity. After dosing the rats were killed, and yellow and blue units were estimated in their livers.

In the ingested carotene each blue unit after treatment with antimony trichloride was associated with 11 yellow units of natural yellow colour. In the livers the number of blue units was usually about the same as had been calculated for the carotene, but the yellow units had been cut down so that each blue unit was now usually associated with only about 0.025 of a yellow unit (Table 16). Confirmation that the virtually colourless chromogen was vitamin A was found in the position of the absorption maximum in the antimony trichloride reaction, which had changed from 590 mμ in the ingested carotene to 620 mμ. Capper[2] collaborated by showing that these changes were accompanied by the appearance in the liver extracts of the absorption band at about 325 mμ in the ultraviolet which is characteristic of vitamin A.

Confirmation of the conversion of carotene to vitamin A was soon reported by Wolff, Overhoff and Van Eekelen,[3] who took small pieces of liver from rabbits by biopsy and then gave the animals injections of carotene. Both carotene and vitamin A were increased in the liver after the injections. Vitamin A was demonstrated in various animal tissues, sometimes together with carotene, but in plants much carotene was accompanied by little or no vitamin A. Modern knowledge, of course, indicates that plant tissues do not contain even traces of vitamin A. Capper, McKibbin and Prentice[4] demonstrated the conversion of carotene to vitamin A in chickens by the same procedure as had been used by Moore for rats.

TABLE 16

EVIDENCE BY MOORE (1930) OF THE CONVERSION OF CAROTENE TO VITAMIN A IN RATS WHICH HAD PREVIOUSLY BEEN DEPLETED OF THE VITAMIN. THE LIVERS OF UNDOSED RATS, KILLED AFTER THE PRELIMINARY DEPLETION PERIOD, CONTAINED NO BLUE UNITS AND NEVER MORE THAN 10 YELLOW UNITS

Daily dose of carotene μg	*No of days dosed*	*Lovibond colour units of carotene ingested by rats*		*Lovibond colour units of vitamin A found in liver*	
		Yellow	*Blue (590 mμ)*	*Yellow*	*Blue (620 mμ)*
750	16	24 000	2200	90	2000
750	22	33 000	3000	110	3300
750	26	39 000	3500	100	3700
750	26	39 000	3500	40	2000

References p 190

Attemps to convert carotene to vitamin A in vitro *or in surviving liver*

Once the derivation of vitamin A from carotene had been proved in the intact animal it seemed that little difficulty was to be expected in effecting the conversion *in vitro* by chemical or biochemical methods Nearly twenty years passed, however, before conversion was achieved, in small yield, by chemical means At the time of writing no biochemical system has yet been devised in which an effective conversion of carotene can be regularly reproduced

Moore [5] had early hopes when he bleached carotene by treatment with benzoyl peroxide, but the continued ability to produce a blue colour with antimony trichloride proved erroneous as a guide to biological activity Later Olcott and McCann [6] observed the appearance of an absorption band at 325 mμ when a colloidal solution of carotene was incubated with ground liver tissues taken from rats deficient in vitamin A They assumed the presence of an enzyme 'carotenase", which could convert carotene into the vitamin In non-biological experiments Bowden and Snow [7] exposed carotene in solution in cyclohexane, and in an atmosphere of nitrogen, to monochromatic irradiation of wavelength 265 mμ Again the appearance of an absorption band at about 325 mμ was claimed to be evidence of the formation of vitamin A

Woolf and Moore [8], however, were sceptical about these claims They emphasised that full spectroscopic data, both in the ultraviolet and in the antimony trichloride action, was necessary for proving that vitamin A had been formed from carotene Thus absorption at 325 mμ, now known to be due to *cis* isomerism, may be shown by carotenoids without their conversion to vitamin A In incubations of liver with carotene these workers could obtain no evidence of the formation of the vitamin When carotene was irradiated in chloroform solution the loss of yellow colour was at first accompanied by an increase in the intensity of the blue colour with antimony trichloride, and a colourless substance absorbing at about 350 mμ was formed More prolonged irradiation caused destruction of both the yellow colour, the blue colour with antimony trichloride and the substance absorbing at 350 mμ Doubtless these experiments would have been carried further if it had been known, at that time, that 350 mμ is near the absorption maximum of vitamin A aldehyde

Against these criticisms v Euler and Klussman [9] supported the view that carotene could be converted by liver tissues in this case from the cow Pariente and Ralli [10] claimed success in one experiment out of four with dog s liver Conversion under very unnatural conditions was also reported later by Wilson, Ahmad and Majumdar [11] who dropped portions of liver from rabbits which had been fed upon carotene into melted paraffin wax After autolysis

for periods of up to 28 days evidence was found of the disappearance of carotene and the formation of vitamin A Willstaet [12] found that carotene itself had no effect on the growth by tissue culture of rat's fibroblasts When a small piece of liver was also added to the medium, however, the growth of the fibroblasts was improved It was presumed that the liver allowed the carotene to act by converting it to vitamin A

In spite of these hopeful reports however, many workers failed repeatedly to observe the conversion of carotene to vitamin A except in the intact animal Thus Ahmad [13] was unable to effect the conversion either by incubating carotene with rat s liver or by perfusing the isolated organ with carotene His only encouragement, apart from his later work with paraffin wax was the detection of a substance resembling vitamin A, together with unchanged pigment in the caecum of rats receiving carotene In experiments to decide whether carotene could be converted by the intestinal bacteria negative results were usually obtained, but in two instances a substance of pale yellow colour was formed which gave a more intense antimony trichloride reaction than the original pigment There was insufficient evidence to identify this substance with vitamin A

Rea and Drummond [14] also failed in attempts to convert carotene by incubating it with liver tissues from vitamin A-deficient rats and cats, and attempts to extract the supposed enzyme 'carotenase' were equally unsuccessful No formation of vitamin A could be demonstrated when a colloidal solution of carotene was injected into the portal vein of a cat They considered that both *in vivo* and *in vitro* much carotene is decolourised without the formation of vitamin A Drummond and McWalter [15] were again unsuccessful with liver incubations, even when the conditions were made more physiological by allowing rabbits to absorb carotene into their livers just before they were killed Later the same authors [16] failed to demonstrate the conversion even by taking lobes of liver from the same vitamin A-deficient rabbit before and after the administration of carotene In contrast to the earlier experience of Wolff and his colleagues they found that the experimental procedure was unsatisfactory the removal of one lobe of the liver was found to cause a marked depression in the vitamin A contents of the others

Even these failures however did little to shake confidence in the view that the liver was the site of conversion As Moore [17] pointed out carotene persisted apparently unchanged throughout the intestinal tracts of rats which in containing noteworthy amounts of unchanged pigment It seemed, at the

References p 190

time, unnecessary to look beyond the liver as the site of the conversion of carotene

The intestinal wall Realisation of the importance of the intestinal wall came at last almost by accident Deuel and his colleagues [18, 19] had studied the problem of producing butter fat rich in vitamin A, and had found that when cows were given massive doses of preformed vitamin A the carotene content of the butter was decreased A similar fall was observed in the carotene level in the blood As a working hypothesis to explain this curious phenomenon it was assumed that the vitamin A might cause an increased concentration of the enzyme "carotenase", or lipoxidase in the liver This in turn would cause an increased discolouration of carotene

In order to put this theory to practical test rats were given carotene by parenteral injection combined with graded doses of vitamin A [20] The carotene was made up either in oily solution, or as colloidal preparations stabilised by lecithin or blood plasma No evidence was obtained that the magnitude of the dose of vitamin A had any effect on the destruction of carotene, but a very interesting side issue was opened up When single or repeated doses of carotene were injected into deficient rats either intraperitoneally, intravenously, or via the spleen the growth responses and times of survival were much less than when the same doses were given orally The livers of rats which died after the injections however, were proved by spectroscopic methods to contain enough carotene to provide oral doses for a year If the conversion of carotene took place in the liver why did the animals die with the pigment readily available for conversion Deuel suggested that in the rat the site of the conversion was not in the liver, but probably in the intestinal wall

Two years later Mattson, Mehl and Deuel [21] reported a direct attack on the problem of role of the intestines in converting carotene Rats were made deficient in vitamin A, were given a single dose of 1800 μg of carotene dissolved in oil and were killed 1–6 hours later Analyses for carotene and vitamin A were made on the liver and on the upper part of the small intestines, which was freed from its contents by washing with saline The results were very irregular but evidence was obtained that vitamin A appeared in the intestines before it appeared in the liver Thus in a rat which was killed 3 hours after dosing the intestines contained a total of 13 5 μg of carotene and 40 3 i u of vitamin A, whereas the liver contained only 1 60 μg of "carotene" and 22 8 i u of vitamin A The interpretation of the results was made difficult by the low concentrations of carotene and vitamin A which had to be estimated This difficulty was increased by the amber colour given by extracts of the livers of deficient animals, which caused high "blank" values

Criticism was also possible on the grounds that no spectroscopic measurements were made, either in the antimony trichloride reaction or in the ultra-

violet region in order to confirm formation of vitamin A We have seen that the loss of yellow colour by carotene even if accompanied by persistence of the antimony trichloride blue colour does not necessarily indicate the formation of vitamin A Spectroscopic measurements were applied however to the small amounts of yellow pigment which were present in the livers of rats and which varied little between the deficient and dosed animals The pigment was certainly not carotene Doubts were cast on Moore s early report of substantial amounts of carotene in the livers of rats given large doses but it was emphasised that the experimental conditions were very different Wiese Mehl and Deuel [22] supplemented the experiments on the intact animal by killing rats just after dosing and incubating their washed intestines They claimed that the incubated intestines contained about 9 i u of vitamin A but nearly half this value was found even if the rats were not given carotene

Some months before the publication of Deuel s papers Morton and his colleagues [23] had made the important discovery that retinene or vitamin A aldehyde is converted to vitamin A in the intestinal wall This at once strengthened the possibility that the conversion of carotene might take place in the same site A preliminary account by Glover Goodwin and Morton [24] of work confirming this view appeared a few weeks before Deuel s detailed reports Vitamin A deficient rats were given massive doses of carotene and were killed a few hours later An extract of the washed intestines was saponified and carotene was removed from the unsaponifiable matter by chromatography From the pooled extracts from four rats enough vitamin A was obtained to prove its identity by the position of the absorption band in the antimony trichloride reaction at 617 mμ and in the ultraviolet at 328 mμ The fact that the yields of vitamin A in different experiments were equivalent to only about 0 1 0 4% of the ingested carotene will give some indication of the technical difficulties involved Later the full account of this work [25] included evidence that the intestines of rats receiving a diet deficient in vitamin A contained no vitamin A even if substantial reserves of the vitamin were present in the liver

Further evidence was obtained by Goodwin and Gregory [26] in experiments on goats Although the blood plasma of this animal is colourless it was found that when substantial doses of carotene were given only 7 19% of the provitamin was excreted in the faeces There seemed a strong *prima facie* case therefore for concluding that the carotene was converted to vitamin A in the intestinal wall In agreement with this view it was found that doses of carotene did not cause any yellow pigment to appear in lymph drawn from a cannula which had previously been inserted into the thoracic duct There was a marked increase however in the vitamin A contents of the lymph It was possible in this way to demonstrate the conversion of carotene to

References p 190

vitamin A in the intestines of an animal which remained alive and conscious Later Alexander and Goodwin [27] succeeded in cannulating rats with fine polyethylene tubing, and demonstrated the conversion of carotene to vitamin A in the intestines of this animal while it also remained conscious

The most thorough and comprehensive experiments on the intestinal conversion of carotene, however, have been described by Kon and his colleagues Their work had proceeded independently of that of Morton, and their first report [28] appeared soon after his preliminary communication In rats and pigs the oral administration of carotene caused the prompt appearance of vitamin A in the intestinal wall and blood plasma (Table 17) In the blood the increased vitamin A was in esterified form Later papers [29, 30] provided a wealth of detail The appearance of vitamin A in the lymph of a pig which had been dosed with carotene was confirmed by ultraviolet spectrophotometry The quick commencement of the conversion was emphasised, even within 5 minutes after giving carotene to rats it was possible to detect vitamin A in their intestines The appearance of vitamin A in the lymph of rats was evident from the yellow fluorescence of the mesenteric lymph ducts under ultraviolet irradiation Vitamin A was at first found after dosing with carotene in both the walls and contents of the intestines By washing out the contents of the intestines while the animals were still alive it was found that most of the vitamin A previously observed in the contents had been transferred from the walls after death When the intestines of rats which had been dosed with carotene two hours previously were cut into small sections the

TABLE 17

EVIDENCE BY THOMPSON GANGULY AND KON (1947) OF THE CONVERSION OF CAROTENE TO VITAMIN A IN THE INTESTINES OF THE RAT

Interval since dosing with carotene	*Blood plasma*	*Vitamin A i u /rat Small intestines (wall + contents)*	*Liver*
Untreated rats	1 3	1 1	1 1
	0 6	0 8	0 5
30 minutes	1 0	8 1	1 3
	0 8	19 2	1 5
1 hour	1 4	21 3	2 9
	1 2	12 8	4 4
2 hours	3 4	22 7	14 3
	4 5	22 8	18 4

Vitamin A was estimated in the blood small intestines and liver of pairs of rats deficient in vitamin A and in rats which had previously been dosed with 2 6 mg of carotene in oily solution Note that the increases in vitamin A in the intestines preceded those in either the blood or the liver

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occurred in the lymph after dosing with carotene was accompanied by an increase in yellow colour, but it could not be clearly established whether the pigment was unchanged carotene or some derivative No carotene appeared after dosing in either the portal or systemic blood of pigs An increase in vitamin A ester preceded an increase of vitamin A alcohol in both circulations

Mattson [31] confirmed the intestinal conversion of carotene in the rat The intestines were digested with alkali and vitamin A was separated from carotene by partitioning between methyl alcohol and light petroleum The vitamin was then adsorbed on a column of magnesium oxide, and its position

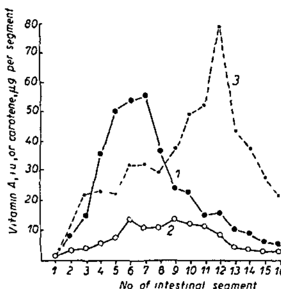


Fig 9 Study by Thompson *et al* (1950) of the concentrations of vitamin A in successive segments of the washed walls of the small intestines of depleted rats which had been dosed 2 hours previously with 4 mg of carotene in oily solution The first segment was taken from the pylorus far enough to include the entrance of the common bile duct and the remaining 15 segments were taken in equal lengths Curve 1 relates to esterified vitamin A curve 2 to vitamin A alcohol and curve 3 to carotene No vitamin A could be detected in the intestines of rats which had not been dosed with carotene

found by its yellow fluorescence under irradiation By removing and extracting the fluorescent portion of the column the ultraviolet absorption band at 328 mμ was observed Cheng and Deuel [32] and Thompson, Coates and Kon [33] demonstrated the conversion of carotene to vitamin A in the intestines of chicks Patel, Mehl and Deuel [34] found that another provitamin, cryptoxanthin, was also converted to vitamin A in the intestines of rats Rosenberg and Sobel [35] claim to have demonstrated the formation of small amounts of vita-

References p 190

min A in isolated small intestines The intestines were removed from rats immediately after doses of carotene had been given and the stomach contents were squeezed into the intestines After incubation for two hours in Ringer solution and extraction, absorption at 328 mμ was observed Similar experiments by other workers [36] [37] have, however, been unsuccessful

The liver reinvestigated In face of the convincing proof that carotene can be converted to vitamin A in the intestinal tract Kowalewski and Henrotin [38] undertook further investigation on the possibility of conversion in the liver Dogs were anaesthetised with Nembutal, and a specimen of blood was taken for the estimation of vitamin A, by the antimony trichloride method from a peripheral vein The liver was then exposed by laparotomy and specimens of liver and of blood from the portal vein were collected An injection of 25 mg of β-carotene emulsified in isotonic glucose was next made into the portal vein and one hour later further specimens were taken from the liver and from the peripheral and hepatic veins In experiments with 6 dogs the injections invariably caused increased concentrations of carotene, as measured by its absorption at 440 mμ, in both the blood and the liver Vitamin A measurements, corrected for the presence of carotene, also indicated substantial increases The following average values expressed in μg of carotene or i u of vitamin A per 100 ml of blood serum or g of liver, were obtained

	Blood Serum				*Liver*	
	Peripheral		*Portal or hepatic*			
	Carotene	*Vit A*	*Carotene*	*Vit A*	*Carotene*	*Vit A*
Before dosing	29 5	214	30 2	214	17 1	81
After dosing	47 4	297	44 6	388	29 7	151

In the absence of spectroscopic confirmation of the formation of vitamin A these findings do not provide complete proof of the conversion of carotene in the liver We may note moreover, that high levels for serum carotene were found even before dosing, which is at variance with general experience that the serum of the dog is almost colourless The pre-experimental diet of Kowalewski's dogs was not rigorously controlled, apart from a preliminary fast of at least six hours

Kowalewski, Henrotin and Van Geertruyden [39] later made further experiments based on their previous pattern but varied by introducing the carotene into the duodenum instead of into the portal vein When the carotene was given as a colloidal suspension increased concentrations of both carotene and vitamin A were found 1–2 hours later in the portal vein, vena cava, and liver, but not in the lymph The substitution of an oily solution of carotene

for the emulsion caused about the same increases of carotene in the blood and liver, but the increases in vitamin A were usually somewhat smaller. After the oily solution had been administered carotene, but not vitamin A, appeared in substantial amounts in the lymph. In this second series of experiments high levels of carotene were again found in the blood of the dogs even before dosing

Conversion after enterectomy or partial hepatectomy

The question of deciding the relative importance of the various possible sites for the conversion of carotene has been left even more open after experiments by Bieri and Pollard [40] on the intravenous injection of colloidal or oily solutions of carotene into entire rats and into rats subjected to various operations Single injections of 100 μg, made without any operation into the tail veins of deficient rats, caused an immediate rise in the level of carotene in the blood plasma, which was followed by a temporary rise in vitamin A After 18 hours the levels of both carotene and vitamin A had fallen to about their original levels, but by this time about 35 per cent of the injected carotene, and about one tenth as much vitamin A, had appeared in the liver In other parts of the body enough carotene could be traced, soon after dosing, to make the total recovery about 70%. In the livers of rats killed 7 days after their injections only traces of carotene, and no vitamin A, could be found

When similar experiments were made on rats from which the small intestines had been removed the formation of vitamin A could still be demonstrated The removal of the kidneys or of 75% of the liver, or ligature of the bile duct, also failed to stop the formation of the vitamin Although the amounts formed were very small, usually of the order of 2 i u., it was claimed that the methods of analyses adopted were accurate enough for the results to be accepted with confidence

Bieri and Pollard concluded that carotene can be converted to the vitamin elsewhere than in the intestines, and suggested that the liver is the main site of the conversion when the provitamin is given by intravenous injection The fall of carotene observed in the liver at increasing times after an injection, combined with the corresponding rise of vitamin A in the blood, was taken as strong support for this view. The discrepancy between this finding and the earlier observations of Sexton *et al* has still to be explained

From all the evidence available, therefore, it appears that the small intestine is the most common site for the conversion of carotene, but that the liver, and probably other tissues, may be alternative sites The quantity of provitamin converted in a given time is limited by factors which are not yet understood but the conversion starts very promptly after a dose of provitamin has been administered.

References p 190

Conversion by chemical methods Success in converting carotene to vitamin A by purely chemical methods was eventually achieved as a sequel to the discovery of retinene$_1$ and its identification as vitamin A aldehyde Since retinene can be reconverted to vitamin A alcohol by chemical reduction it was obvious that the conversion of carotene to retinene was all that was needed for a conversion in two stages The necessary step was accomplished with very small yield by Hunter and Williams [41] who oxidised the pigment with hydrogen peroxide Later Meunier Jouanneteau and Zwingelstein [42] claimed a much more efficient procedure which was based on the finding of Ball Goodwin and Morton [43] that vitamin A is oxidised to retinene in the presence of manganese dioxide When carotene was submitted to the same treatment by agitation with the dioxide in ethereal solution in the presence of air a yield of 60–80% of retinene was claimed Unfortunately confirmation of the efficiency of the method in the hands of other workers has not been forthcoming

According to Hunter [44] the oxidation of carotene by hydrogen peroxide produces not only retinene itself but also higher homologues Recently Glover and Redfearn [45] have confirmed this finding which suggests that the carotene molecule is oxidised stepwise from its end rather than cut in the centre The high yields claimed by Meunier of course would imply that under his experimental conditions central cleavage predominated It seems probable that the point of oxidation may be different according to whether the carotene molecule is in solution or is adsorbed on a surface In the animal it seems possible that the metabolism of carotene may be divided between both paths

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CHAPTER 18

The Absorption of Preformed Vitamin A

As already mentioned preformed vitamin A is absorbed much more efficiently than its provitamins A liver reserve of vitamin A which would require week or months to build up from a diet containing carotene can be acquired within a few hours when a massive dose of vitamin A is ingested

In consequence of its more efficient absorption preformed vitamin A doe not usually accompany its provitamins in the faeces It may be excreted however after massive dosing and particularly in infants It is normally absent from the urine in the human and most other species but may be excreted by this route under special circumstances (see Chap 32)

It will be convenient to discuss first the paths of absorption of the vitamin and its changes between the free and esterified form We can then proceed to the more extensively investigated problem of the quantitative efficiency of absorption of the vitamin

Mode of Absorption

The lymphatic route In their interesting observations on a patient with a diversion of the thoracic duct Drummond Bell and Palmer[1] found that preformed vitamin A in contrast to carotene was very efficiently absorbed by this route Later work has included experiments by Eden and Sellers[2] on bullocks sheep and rats In animals killed soon after massive doses of vitamin slight increases were found in both the systemic and portal blood but much larger increases were observed in the intestinal lymph Thus the following mean values in i u per 100 ml of fluid were found in the blood and duodenal lymph of dosed and undosed bullocks

	Systemic blood	*Portal blood*	*Lymph*
Undosed	83	90	225
Dosed	162	147	1500

Similar findings were made in the other animals The lymph in other parts of the body, as might have been expected remained low in vitamin A Unless the superficial picture must be modified, to allow for differences in the rates of flow of lymph and blood, it would appear therefore that the lymphatics are more important than the portal vein for the absorption of the vitamin The small superiority in the vitamin A level after dosing found for the sytemic over the portal blood would certainly favour this conclusion, if significant.

An interesting qualitative study of the absorption of vitamin A by the intestinal lymphatics was made by Popper and Volk [3] Frozen sections of the intestines of rats which had been given massive doses of natural vitamin A esters were taken at ten different levels, and were examined by fluorescence microscopy The presence of vitamin A in the lymphatics could be demonstrated by its fading green fluorescence within 25 minutes after dosing Absorption took place throughout the whole small intestines, but reached its peak at the border between the upper and middle thirds

Hydrolysis and esterification During absorption esters of vitamin A are at least partially hydrolysed in the intestinal wall but are re esterified before they appear in the blood during the temporary increase which follows absorption Gray, Morgareidge and Cawley [4] examined the changes undergone by vitamin A esters distilled from fish-liver oil after ingestion by rats Analytical distillation of extracts of the gastro-intestinal walls indicated that at 220 minutes after dosing 59% of the total vitamin recovered was in the form of the free alcohol and that at 400 minutes the proportion had risen to 82% In experiments on calves and sheep however, Eden and Sellers [5] obtained somewhat different results Partial or complete hydrolysis of esters as indicated by chromatographic analysis, usually occurred in the intestinal lumen In contrast the vitamin in the intestinal walls was predominently esterified Thus in calves which had been dosed with vitamin A esters 73% of the vitamin in the walls was esterified, and in sheep 56% Even after dosing with vitamin A alcohol it was found that esters predominated in the walls The increased vitamin in the lymph, moreover, was almost entirely in the ester form In spite of differences in detail for the findings in rats and ruminants it is clear that changes from the free to esterified form readily take place during the absorption of the vitamin

The importance of bile Evidence that bile is necessary for the absorption of vitamin A may be found in the early observations of Altschule [6] on infants who had died from chronic atresia of the bile ducts Microscopic evidence of epithelial lesions typical of vitamin A deficiency, was found at autopsy in about half the cases Since the diet was adequate it appeared that there was a failure in the absorption of the vitamin

On the other hand observations by Greaves and Schmidt [7] on vitamin A

References p 206

deficient rats which have been made jaundiced, either by poisons or by ligature of the bile ducts, suggest that the failure in the ability to absorb the vitamin is not complete Thus in contrast with the failure of carotene to correct the keratinised vaginal smears in such animals treatment with preformed vitamin A was found to be effective

Recently Bernhard, Ritzel and Steiner[8] have claimed that the bile pigments, bilirubin and biliverdin, act as antioxidants for vitamin A In rats with lymphatic fistulas, but intact bile ducts, about 50% of a single oral dose of 3300 i u of vitamin A was absorbed from the intestines into the lymph Diversion of the bile reduced the absorption to only about 1%. In animals so treated an improvement to 20% absorption could be secured by giving natural bile, but the administration of taurocholic acid only improved the absorption to 2–10% The difference was attributed to the presence of bile pigments in the natural bile *In vitro* the pigments protected the vitamin from oxidation, and also retarded the formation of peroxides by linoleic acid

The Efficiency of Absorption

A few workers have compared the efficiency of absorption of vitamin A in various forms of chemical combination, or in different vehicles, by measuring the rates of growth induced in experimental animals by low or moderate doses Thus Halpern, Biely and Hardy[9] inferred from superior growth that chicks could "utilise" the vitamin better from fresh gray fish liver oil than from stale and better from an aqueous emulsion than from an oily solution

The two most popular procedures, however, have been based on measurements of vitamin A in the liver or in the blood plasma after the administration of large doses Of these procedures measurements of liver stores must obviously give the most reliable information It is necessary, however, to use animals which can first have their liver reserves of vitamin exhausted, and which can be killed after dosing with the source of vitamin under investigation The results so obtained, moreover, may not be transferable from one animal to another The second procedure based on blood levels, therefore, is useful in investigations on human subjects

Liver storage tests An early study by Baumann, Rising and Steenbock[10] indicated that when vitamin A was administered to rats only 10–20% of the dose could be recovered from the liver Soon afterwards, however, Golding, Kon and Campion[11] reported a recovery of 47% in pigs At first sight this disparity might be attributed to the difference in species but later it was shown by Lemley, Brown, Bird and Emmett[12] that the efficiency of absorption is profoundly influenced by the magnitude of the

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depleted rats were dosed for 3 days with 63 i u of vitamin A they stored 11% When the dose was raised to 4000 i u daily the efficiency of storage was 38% but a further increase to 80 000 i u daily reduced the efficiency to 13% Numerous intermediate rates of dosing gave efficiences which followed con sistent pattern (Table 18)

TABLE 18

THE INFLUENCE OF THE DAILY DOSE ON THE STORAGE OF VITAMIN A IN THE LIVERS OF RATS THE DOSES WERE GIVEN AS AN OILY DISTILLATE FOR 3 DAYS (LEMLEY *et al* 1947)

Daily dose i u	*Average total vitamin A in liver i u*	*Storage %*	*Daily dose i u*	*Average total vitamin A in liver i u*	*Storage %*
63	21	11 1	8000	8000	33 3
125	92	24 5	16000	13900	29 0
250	215	28 6	32000	21200	22 1
500	480	32 0	48000	25400	17 6
1000	1032	34 4	64000	29950	15 6
2000	2220	37 0	80000	31800	13 3
4000	4530	37 8	118400	49200	13 9

Davies and Moore [13] agreed with these findings in principle but found even less efficient storage at very low doses and more efficient at high doses Thus with single doses of 100 200 400 and 600 i u given as halibut liver oil 48 hours before killing the efficiencies of storage were 0 13 15 and 38% With a massive dose of 60 000 i u the absorption rose to 80% but when 30 000 i u was given daily for 25 days the absorption was only 10% It ap peared therefore that at low doses about 100 i u of the vitamin was lost before storage in the liver commenced The percentage of storage then in creased with the magnitude of the dose although it must be noticed that the actual amounts of vitamin A which were lost became greater Finally if re peated high doses were given the percentage stored was reduced presumably on account of saturation of the liver with vitamin

More extensive experiments by Moore Sharman and Ward [14] confirmed and extended these results Groups of rats of both sexes were given graded daily doses of vitamin A acetate from 10 to 10 240 i u for 5–6 weeks They were then killed and vitamin A was estimated in the liver and kidneys The efficiencies of storage calculated for the liver and kidneys combined and averaged between the sexes ranged from 2% or less at daily doses of 10 i u up to 80% at doses of 640 i u (Fig 10) The examination of the kidneys was undertaken because work by Johnson and Baumann [15] had shown that when the level of dosing is low they may contain more vitamin A than the liver

References p 206

Thus at doses up to 20 i u in females or 40 i u in males, but not at higher doses, the contribution by the kidneys to the total absorption would have been too large to neglect The disappearance of vitamin at low levels of dosing observed in previous work can be explained partially, but not entirely, by failure to examine the kidneys

Discussion of the interesting effects of sex on the storage and distribution of the vitamin may conveniently be postponed for discussion later (Chap 35)

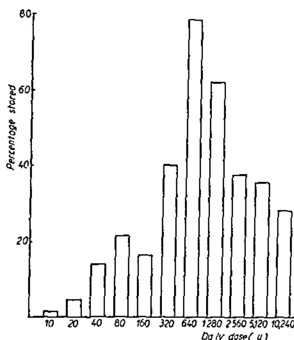


Fig 10 The effect of the magnitude of the dose on the storage of vitamin A by rats The daily doses indicated were given as vitamin A acetate in oily solution for periods of 4 to 6 weeks (after Moore Sharman and Ward 1951)

Storage tests with vitamin A alcohol and various esters Comparisons of the absorption of the vitamin in various forms of combination were made by the liver storage method by Week and Sevigne [16] When groups of depleted chicks were given 30 000 i u of vitamin A dissolved in corn oil, and supplied as either the free alcohol, the acetate or undistilled natural esters, the mean efficiencies of storage were 35 6 33 6 and 23 8% It will be seen later that the differences between the groups were emphasised when other oils were used as diluents For groups of rats, which were dosed with 9000 i u of vitamin A the efficiencies of absorption were 33 2% for the alcohol, 36 1% for the acetate and 29 2% for natural esters

Blood tests as a measure of absorption The increase in vitamin A in the blood plasma observed at a chosen interval after dosing represents only a small fraction of the total amount which has been ingested It is impossible, therefore to measure the efficiency of absorption directly, and we must be content with comparing the absorption

of the vitamin from different sources This is only possible, moreover, on the assumption that the total absorptions are proportional to the increases observed in the plasma Fortunately this assumption may often be justified, but various possible complications are readily visualised Thus different media containing the vitamin may be digested at different rates, and the peak of absorption may not always occur at the same interval after dosing Repeated observations at different intervals and the comparison of the areas under the absorption curves rather than the height of a single peak, may partially overcome this objection It remains possible, however, that slow removal of the vitamin from the blood plasma by the liver may give the impression of rapid absorption from the intestines In healthy subjects this complication probably seldom arises but caution must be exercised on the interpretation of tests on pathological subjects (see Chap 32)

Week and Sevigne [17] used blood examinations to compare the absorption of vitamin A alcohol, acetate and two types of natural esters by healthy human subjects Groups of 18 males and 17 females were dosed at intervals of a few days in different orders with 440,000 i u of the vitamin in the various forms The results were more consistent in specimens taken at different intervals for males (Fig 11) than for females At the "peak" intervals of 5 hours the increases in males were 2480 i u per 100 ml for the alcohol, 2170 i u for the acetate 1970 i u for undistilled natural esters and 1780 i u for distilled natural esters In the females the corresponding values were only 1440 1080 970 and 1340 i u This sexual difference will be remembered later (Chap 35)

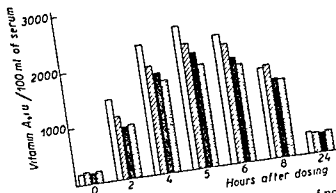


Fig 11 Average vitamin A levels in the blood serum of a group of normal men before, and at various intervals after the ingestion of a single dose of 440 000 i u of vitamin A in various forms (after Week and Sevigne 1950)

- Vitamin A Alcohol
- Vitamin A Acetate
- Vitamin A Natural Esters (undistilled)
- Vitamin A Natural Esters (distilled)

p. 206

If we venture to compare the above results with those found by liver storage tests in the chick and rat we find the following orders for the efficiency of absorption

Human male	1 Alcohol	2 Acetate	3 Undist Nat Esters
Human female	1 Alcohol	2 Acetate	3 Undist Nat Esters
Chick	1 Alcohol	2 Acetate	3 Undist Nat Esters
Rat	1 Acetate	2 Alcohol	3 Undist Nat Esters

FACTORS AFFECTING THE EFFICIENCY OF ABSORPTION

The absorption of preformed vitamin A is influenced by many factors. Some act by altering the stability of the vitamin, others by aiding emulsification and yet others by affecting the activity of the digestive processes In the human, emulsification is probably the most important of these factors Although its investigation has been only a recent development it will be convenient to give it priority in our account

Emulsifying agents The effect of agents of the Tween type was studied in 1947 by Kramer, Sobel and Gottfried [18] in attempts to improve the assimilation of the vitamin by children with impaired intestinal absorption (see Chap 32) When the children were dosed with vitamin A in oily solution there was no increase in the blood after dosing but a significant increase followed after dosing with an aqueous emulsion of the vitamin made with a polyalkylene derivative of sorbitan monolaurate Moreover in normal children the rise in the blood after dosing was greatly enhanced by emulsification (Fig 12) Research in the same year by Lewis Bodansky, Birmingham

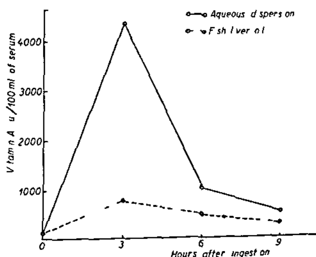


Fig 12 Vitamin A in the blood of a normal child before and after dosing on separate occasions with 6000 i u of vitamin A per lb of body weight either in oily solution or as an aqueous dispersion (after Kramer *et al* 1947)

and Cohlan [19] gave similar results In this work the emulsions were made with Tween in conjunction with 47% propylene glycol or glycerol From Table 19 it will be seen that emulsification increased the apparent efficiency of absorption at all ages, although the efficiency was lower in infants than in adults for both the oily solution and the emulsion

TABLE 19

EFFECT OF EMULSIFICATION WITH TWEEN ON THE INCREASE OF VITAMIN A IN THE BLOOD PLASMA OF HUMAN SUBJECTS 5 HOURS AFTER DOSING (LEWIS *et al* 1947)

Subjects	*No*	*Dose* *i u*	*Mean increase in blood i u /100 ml* *Oily solution*	*Aqueous emulsion*
Premature infants	12	35 000	64	274
Full term infants				
1–3 months	6	35 000	200	1 000
Children 7–11 years	4	5 000/kg	600	4 000
Adults	6	500 000	1 750	4 500

An interesting study by Lewis and his colleagues on the faecal excretion of the vitamin however indicated the necessity of caution before accepting the increase in the plasma as a direct measure of absorption Thus from Table 19 it might be calculated that the infants 1–3 months old absorbed the vitamin from the aqueous emulsion more efficiently than from the oily solution in the ratio of plasma increases *i e* 1000 to 200 or 5 to 1 The analysis on the faeces however indicated that the average absorption of the vitamin was about 93% for the aqueous emulsion and 62% for the oily solution This would suggest that the absorption was only some 50% better from the emulsion than from the oil It seems highly probable however that more vitamin would be destroyed in the intestines with the oil than with the emulsion and that the efficiency of absorption with the oil may therefore be considerably lower than the faecal analysis would indicate Probably the true ratio for the efficiencies of absorption lies somewhere between the values reached by the two methods

Experiments with animals by the same workers confirmed the superior absorption from aqueous emulsions When young rats were dosed with 13 000 i u of an oily solution of the vitamin by stomach tube and were killed 3 6 or 24 hours later the levels in the plasma were 900 1015 and 120 i u, 23% of the vitamin was lost in the faeces and 30% was stored in the liver (24 hours) When the same dose was given as an emulsion the levels in the blood were 2460 2010 and 310 i u only 5% was passed into the faeces and 58% was stored in the liver Equally impressive results were obtained with

References p 206

guinea pigs Sobel and his colleagues [20] recognised the limitations of the level of vitamin A in the blood as a measure of absorption, and supplemented their observations on humans with proof that rats stored three times more vitamin A when given an emulsion of the vitamin than when given an oily solution

The significance of these results seems beyond question, but it remains puzzling why Moore, Sharman and Ward [14] should have found up to 80% absorption of vitamin A as measured by liver storage, in rats given the vitamin in oily solution Possibly the cause of this higher absorption may lie in a longer interval between dosing and killing or in the use of older rats

Pressure of space prevents discussion of many other admirable papers on the effect of emulsification on the absorption of vitamin A by humans [21–28] and animals [29–33]

Lecithin Emulsification of lecithin has also been reported to aid the absorption of vitamin A, but the effect is less striking and less well authenticated, than with Tween Adlersberg and Sobotka [34] found that doses of 10–25 g of commercial lecithin increased the absorption of both fat and vitamin A in human subjects Thus 4 hours after doses of 120,000–180 000 i u of vitamin A the mean increase in the plasma was 31 i u per 100 ml without lecithin and 153 i u with lecithin Kern, Antoshkiw and Maiese [35] also found that lecithin increased the absorption of vitamin A in 6 out of 7 human subjects Slanetz and Scharf [36] reported that the addition of 1% of soya bean phosphatides to the diet increased the growth of rats given small doses of cod liver oil Patrick and Morgan [37] claimed that chicks required a factor for the efficient utilisation of vitamins A and E which could be supplied as 2% of soya bean phosphatides, or as 5% of dried yeast

The small improvement in the growth responses of rats to low doses of vitamin A caused by the addition of lecithin was confirmed by Esh and Sutton [38], who also observed an increase of some 20% in the storage of the vitamin Aschaffenburg *et al* [39] found that soy bean lecithin sometimes improved the storage of vitamin A in calves

The significance of many of these findings however, was questioned by Guerrant and Thompson [40] In their experience the potency of certain specimens of lecithin in supporting the action of small doses of vitamin A could be concentrated in the unsaponifiable fraction and was associated with the presence of carotenoid pigments It would still be difficult to explain however, how the presence of small amounts of provitamins in lecithin could facilitate the absorption of massive doses of vitamin A

Vitamin E Moore [41] noticed that the vitamin A reserves of rats which had been kept for long periods on diets deficient in vitamin E were invariably lower than in rats which had been dosed with vitamin E The results of a typical experiment are given in Table 20, and in others differences

of 2–10 fold between the groups adequate and deficient in vitamin E were found As will be shown later (Chap 20) advanced deficiency of vitamin E

of the liver rather than in the absorbing power of the intestines

The protection of vitamin E on preformed vitamin A during its absorption from the intestinal tract however, was studied later by Hickman and his colleagues [42] in experiments which were parallel to those on carotene (see Chap 16) Mixed tocopherols the individual α-, β and γ tocopherols lauryl hydroquinone, ascorbic acid and palmityl ascorbic acid all improved the growth responses of rats given small doses of vitamin A In agreement with their lack of antioxidant power *in vitro* tocopherol esters were less effective in aiding growth than the free alcohols

The effect of mixed tocopherols both in aiding growth and in increasing the storage of vitamin A in rats was confirmed by Lemley *et al* [43] They emphasised however, that the action of tocopherol could only be demonstrated under suitable experimental conditions Thus growth was aided at low dosages of vitamin A but not at high doses Liver storage of vitamin A was not influenced when this vitamin was given with or without tocopherol for only three days but considerable differences were found when dosing was continued for several months

It seems probable that in short term experiments vitamin E can increase growth by stabilising small doses of vitamin A in the intestines, even in

TABLE 20

THE EFFECT OF GRADED DOSES OF DL α TOCOPHEROL ON THE STORAGE OF VITAMIN A IN FEMALE RATS KEPT FOR 24–29 WEEKS ON A DIET DEFICIENT IN VITAMIN E AND SUPPLEMENTED WITH 1000 I U OF VITAMIN A WEEKLY A BROWN UTERUS INDICATED THAT THE DOSE OF TOCOPHEROL WAS INADEQUATE (MOORE, 1940)

Weekly dose of tocopherol mg	*Colour of uterus*	*Vitamin A i u /g liver*	*Mean Vitamin A*
3	white	1100	1300
3	white	1500	
1	white	1200	1200
1	white	1200	
0 3	brown	650	600
	brown	550	
0 1	brown	430	495
	brown	560	
nil	brown	280	395
	brown	510	

animals which have not been made deficient in vitamin E Wide differences in the storage of vitamin A however probably only occur in long term experiments when rats which are adequate in vitamin E are compared with severely deficient animals Failure to observe any influence by vitamin E may be due to the choice of unsuitable timing and dosing levels [44] or to the use of basal diets which are not deficient in vitamin E [45] In early work by Bacharach [46] in which rats were kept on a basal diet deficient in vitamin E for the comparatively short period of 14 weeks dosing with vitamin E caused an improvement of only about 50% in the storage of vitamin A

Other antioxidants Although only the tocopherols appear to accompany

other sources The scope of such protection probably extends to the intestinal lumen and will often enable a larger proportion of the vitamin to be absorbed The protective role of antioxidants *in vitro* has already been mentioned (Chap 9)

Dietary or solvent fat Russell *et al* [47] concluded that the influence of a diet low in fat in depressing the absorption of carotene was not extended to vitamin A The nature and freshness of the fat however are obviously important Thus Halpern and Biely [48] found that the growth responses of chicks to specimens of grayfish liver oil were usually reduced either by oxidative treatment which was too mild to destroy the vitamin or by admixture with an oxidised vegetable oil In the same species Week and Sevigne [16] found decided differences in the storage of vitamin A given as natural esters according to the vegetable oil which was used as diluent With corn oil the efficiency of storage was 23 8% and with castor oil 17 0% with ethyl laurate 12 2% and with jojoba seed oil only 8 1% The range of values with vitamin A alcohol as the source however was much narrower with efficiencies of 35 6 27 4 29 4 and 32 8 respectively

The effect of rancid fats in destroying vitamin A in the food during digestion was studied in interesting early experiments by Lease Lease Weber and Steenbock [49] The destruction was measured indirectly by estimating the vitamin in the livers of rats which had been given a source of vitamin A in addition to the fat under investigation The admixture of rancid fats ozonised fats or palmitic peroxide with the food caused the destruction of vitamin A or its precursors given in various forms Partial destruction occurred with fats previously heated to temperatures reached in domestic cooking Vitamin A was not destroyed by various oxidation products of glycerol pyruvic acid propyl aldehyde straight chain aldehydes and methyl ketones of 7 12 carbon atoms decomposition products formed by the commercial hydrogenation of fats or by fresh fats heated in the absence of oxy

gen The destructive powers of rancid fats could not be removed by steam distillation or extraction with alcohol, but were reduced by heating, which also lowered the peroxide value Ascorbic acid, hydroquinone, gallic acid and ethyl gallate all failed to neutralise the destructive power of rancidity Vitamin A was not destroyed in animals fed upon a diet containing rancid fats if a long enough interval was allowed after the last meal for the stomach to become empty After a meal of rancid fat, however, peroxides persisted in the stomach for 4 hours, and the vitamin was destroyed if it was given, even as a separate dose, during this period.

Miscellaneous factors Further interesting possibilities have been suggested by isolated communications on the effects of various widely different substances on the absorption or storage of vitamin A According to Braude *et al* [50] the use of dried yeast as the main source of protein for pigs caused an increase in the size of the liver and a decrease of some 50% in the stores of vitamin A High and Day [51] found that the absorption of preformed vitamin unlike that of carotene, was not affected by large supplements of squalene or phytol Burgess *et al* [52] reported that the addition of penicillin to the diet of chicks increased vitamin A in their blood and liver. In rats given 7 5 µg of vitamin A daily, however, Hartsook *et al* [53] found that aureomycin had little effect on the small stores of vitamin which were accumulated

The drug atropine was found by Ingefinger Moss and Helm [54] to reduce the absorption of vitamin A by human volunteers, as evidenced by the complete or partial suppression of the increase in the blood after massive dosing with the vitamin They commented that a reduction of the motor activity of the small intestine, such as is caused by the drug, is also a feature of sprue, a disease in which the absorption of the vitamin is seriously impaired Guggenheim [55] reported that when rats were given prolonged treatment with atabrine the anti malarial drug the capacities of their livers to store vitamin A was adversely affected Even toxic levels of atabrine, however, did not seem to accelerate the appearance of signs of deficiency in rats which were deprived of the vitamin

Points of detail in pharmaceutical or factory practice may be important Thus Sobel and Rosenberg [56] have shown that the efficiency of absorption of vitamin A by humans from capsules is greatly affected by the material used The best absorption was found with a capsule which disintegrated slowly under the action of digestive juices *in vitro* Laughland and Phillips [57] found that the use of 25% of sodium bentonite, a clay, as a binding agent for pelleted animals' food caused serious destruction of vitamin A The vitamin was adsorbed upon the clay and converted into inactive anhydrovitamin A (see Chap 11)

References p 206

Parenteral Absorption

When vitamin A in oily solution is injected parenterally by any route its utilisation measured either by growth response or by storage in the liver is usually much le[illegible]
emulsifying age[illegible]
allow rather more efficient utilisation of the vitamin than is possible with oily solutions

Injections Groth and Skurnik[58] injected vitamin A which had been emulsified with sodium oleate either intramuscularly or intravenously into rabbits and humans In rabbits intramuscular doses of as much as 180 000 i u had little effect on the level in the plasma Probably assimilation was slow from the site of injection into the blood stream Intravenous injections of 36 000–180 000 i u caused the level in the plasma to be doubled but only a fraction of the total dose could be found in the blood Since the vitamin was not delayed at the intestinal wall but was added abruptly to the blood stream much larger increases might well have been expected In the human intravenous injections of 60,000 i u failed to influence the level in the plasma even if the blood was collected a few minutes after the injections It was assumed that the removal of the vitamin from the blood stream by the tissues is very rapid

With[59] found that in rats guinea pigs and hens subcutaneous and intramuscular doses of vitamin A in oily solution were transferred to the liver less rapidly than when they were given by mouth At autopsy more vitamin was recovered in oily solution at the injection site than in the liver According to Lemley *et al*[12] the efficiency of absorption of vitamin A when given in oily solution by intramuscular injections was only 2% of the efficiency obtained in oral dosing Subcutaneous injections were more effective with 35% of the efficiency found for the oral route

Aqueous dispersal with Tween was claimed by Kramer Sobel and Gottfried[18] to increase the efficiency of vitamin A when given by intramuscular injection into sick children but only a few cases were studied with rather inconclusive results In lambs Bolin Eveleth and Bolin[60] found by analysis on liver obtained by biopsy that aqueous emulsions of vitamin A were absorbed just as well when they were given by intravenous injection as when they were given by mouth In contrast to the findings of Groth and Skurnik[58] very high concentrations of vitamin were found in the blood a few hours after intravenous injections Peak values were over 2 000 i u per 100 ml as against only 110–270 after oral dosing Intramuscular injections combined with hyaluronidase to cause the spread of the injected fluid into the tissues were also effective storage in the liver was as good as after intravenous injections

and peak values of over 1000 i u were found in the blood Possibly parenteral routes for the administration of vitamin A may have a special advantage in ruminants in avoiding the necessity of the vitamin being mixed with the contents of the rumen

Absorption through the skin The absorption of vitamin A through the skin has attracted little interest, probably because aqueous dispersions can usually be well absorbed by the oral route even when the digestion of fat is disturbed. As early as 1937, however, Aykroyd and Wright [61] mentioned that keratomalacia may be cured in infants by the application of cod liver oil on a binder Recently Sobel *et al* [62] succeeded in curing vitamin A deficiency in rats by rubbing cod liver oil or a proprietory preparation of vitamin A upon a shaved area in the scapular region Precautions were taken against the vitamin being accessible to the mouth Small amounts of vitamin were found subsequently to have been transferred from the skin to the liver and kidneys

TABLE 21

FACTORS WHICH INFLUENCE THE ABSORPTION AND STORAGE OF PREFORMED VITAMIN A *

Increased absorption	*Decreased absorption*
Moderately high rate of dosing	Low or very massive dosing
Maturity	Infancy
Aqueous emulsification (particularly if dosing by injection)	Oily solution (particularly by injection)
Bile bile pigments	Diversion of bile duct
Freshness of oily medium	Staleness and rancidity of oily medium
Vitamin E and other antioxidants	Pro oxidants and fats which promote oxidation
Administration as alcohol or acetate	Administration as natural esters (slight effect only)
Lecithin penicillin(?)	Atropine atabrine adsorption on bentonite clay

* Some of these factors only operate in appropriate circumstances For example aqueous emulsification will influence the absorption of oral doses of the vitamin A by human infants but not always by rats which can usually absorb the vitamin very efficiently from oily solution For effects of disease and sex see Chapters 32 and 35 respectively

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CHAPTER 19

The Storage and Distribution of Vitamin A in the Body

The distribution of vitamin A in the body has many points of interest Thus we are all familiar with the role of the liver, under normal conditions of [illegible] The [illegible] n in [illegible] ing, however, on the particular properties of either the vitamin or the liver tissues, which make this vast storage possible Vitamin E, another alcohol which resides in the unsaponifiable fraction of oils, is fairly evenly distributed throughout the body, with perhaps its greatest concentrations in the fat depots The reticulo-endothelial system, which is largely responsible for holding vitamin A in the liver, is also found in the spleen and in bone marrow, from which the vitamin is virtually absent.

After the liver the kidneys probably come next in importance as a site for the storage of vitamin A They may even hold higher concentrations than the liver when the level of dosing is low (Chap 35) Other sites in which [illegible] and body fat

and sometimes even for a particular individual in spite of dietary fluctuations Presumably *this control depends mainly on the power of the liver to absorb* or give out vitamin as required

There is of course ample evidence that vitamin A, or its aldehyde retinene, is present in small amounts in the retina of the eye (see Chap 22) The detection of the vitamin in this site followed clinical observations that failure in dark adaptation is associated with a lack of vitamin in the diet In many of the other sites which become injured during deficiency of the vitamin, however, there has been no clear demonstration that the vitamin, under normal conditions of nutrition, is present in more than traces

Distribution throughout the Body

Experiments with rats Most of our *information* on the concentration of vitamin A in the various organs has been gained in

studies on rats Unfortunately the most extensive of these investigations were made before modern chromatographic and photoelectric methods of estimation had been developed Further research, with these refinements available might well reveal the presence of small amounts of vitamin in sites which have hitherto been overlooked

In 1925 Sherman and Boynton [1] applied biological tests to the organs of rats which derived their vitamin A from the inclusion of 33% of dried milk in their diet The daily doses necessary to promote growth in other rats were 0 02 g of liver 0 1 g of lung, 0 1 g of kidney or 4 0 g of muscle Neglecting any vitamin in the adipose tissues and skin they concluded that the liver contained 90% of the body s stores of vitamin From non quantitative observations by the antimony trichloride test Kerppola [2] reached a similar conclusion on the relative amounts of vitamin in the liver and lungs A positive reaction was also found for the intestines and their contents but all the other organs which were examined gave negative results Moore [3] used the antimony trichloride reaction, with the aid of a Lovibond Tintometer, in studies of vitamin A in the tissues of rats which had been given liberal amounts of carotene By this treatment total stores of up to 50 000 i u of vitamin A were accumulated in the liver Apart from the intestines, which contained much yellow pigment the rest of the carcase contributed only about 100 i u of vitamin A When the various tissues were examined separately the intraperitoneal fat contained 0, 5 5 and 5 i u in four experiments and the kidneys and lungs each 0 0 and 5 i u in three experiments No vitamin could be detected in the brain heart pancreas spleen suprarenals, thymus or testes The skin and bones gave feeble red colours

These findings confirmed the predominance of the liver in storing the vitamin Thus the concentration of vitamin in the liver fat was about 100 000 times greater than in the intraperitoneal fat The method of analysis, however was too crude for an adequate examination of the small amounts which are located in the rest of the body Even in such familiar sites as the blood, eyes and suprarenal no vitamin could be detected

In further experiments Davies and Moore [4] estimated vitamin A in the organs of rats which had been given either carotene or the preformed vitamin By reducing the final volume of the extracts smaller amounts of vitamin could be detected than in the previous work Rats which had been dosed with carotene until their livers contained 30–90 i u vitamin A per g had 1–3 i u of vitamin A per g in their kidneys Similar amounts were found in the lungs of two out of five animals but not in the remaining three animals By giving vitamin A in large doses, but still below the toxic level concentrations of 25 000 i u per g liver, 30 i u per g of kidney, and 250 i u per g of lungs were attained The intraperitoneal fat deposits had 50 i u per g and

References p 219

the suprarenal glands 1500 i u per g Traces of the vitamin appeared in the pancreas, thymus and spleen By raising the dose rate still further, and into the toxic region, the liver contained up to 36,000 i u per g, the kidneys 360 i u , lungs 600 i u and suprarenals 30 i u. Concentrations of up to 15 i u per g were found in the muscles, heart, spleen and brain

It was concluded from these experiments, therefore, that liver is normally the main storage site over a wide range of dosing. With moderate intakes of vitamin A or carotene much lower concentrations are regularly present in the kidneys, and sometimes in the lungs Heavy dosing with vitamin A increase he concentration of vitamin up to 100 times the level typical of the animals in the wild state, and brings the kidneys and lungs up to concentrations which would be considered typical of liver in normally fed rats Saturation point for vitamin A is difficult to recognise, but the possibility of saturation may be assumed from the failure of the liver to store repeated high doses (see Chap 18) Since concentrations of over 20,000 i u can be reached under experimental conditions it must be concluded that under normal conditions of nutrition only the livers of polar bears and of certain marine animals ever approach saturation

Later confirmatory evidence of these conclusions was obtained by the fascinating technique of fluorescence microscopy From Chapter 7 it will be recalled that vitamin A had a strong greenish yellow, fading fluorescence when it is exposed to ultraviolet irradiation Van Querner[5] first used this property as a means of visualising the position of vitamin A in frozen sections of tissues The fluorescence was seen in sections of liver, suprarenal and pituitary glands and in the retina Later more extensive studies, confirming the presence of the vitamin in the liver, lungs, kidneys and suprarenals were made by Popper[6, 7]

In the author's own experience it is possible, without cutting sections to recognise by their fluorescence at autopsy those rats which have been given liberal doses of vitamin A In the liver and kidneys the yellow fluorescence is usually damped by haemoglobin, but it may be clearly observed in the intra peritoneal fat and in the suprarenals The level of dosing necessary to promote fluorescence, however is usually about 150 i u daily[8], which is far greater than the allowance necessary for normal health and growth Normally nourished animals, therefore, often fail to give any clear indication of the presence of vitamin A by this method

The introduction of the photoelectric absorptiometer made it possible to measure low concentrations of vitamin A, as in blood, with reasonable accuracy In the rat, as in other species, much more vitamin is present in the plasma than in the cells For normal plasma 80 i u per 100 ml, or roughly 0 8 i u per g, might be taken as a typical reading The resting level in the

usually contained more vitamin than the liver, both in concentration and in total amount

According to Edisbury, Lovern and Morton [16] the eels *Anguilla vulgaris* and *A aucklandi* Rich also have a high ratio of non-hepatic to hepatic vitamin A Thus the livers were found to contain 20–60% of the total, body 25–65%, skin about 10%, viscera 2% and head 1½% The concentration in the body increased with age, and body oils approaching cod-liver oil in potency were sometimes obtained In herrings biological tests by Scheunert and Schieblich [17] suggested that the vitamin was mainly present in the gonads, and was more concentrated in females than in males

The eyes are a main site of accumulation of vitamin A in various marine invertebrates According to Wald [18] the vitamin is present in large amounts in the eyes of cephalopod molluscs and some arthropods Fisher, Kon and Thompson [19] made the remarkable observation that practically the whole vitamin A contents of *Meganyctiphanes norvegica*, and of certain other shrimps are concentrated in the eyes The picture in Crustacea, however, is not always so one sided Thus Neilands [20] certainly found that the lobster

TABLE 22

POINTS OF INTEREST IN REGARD TO THE DISTRIBUTION OF VITAMIN A IN ANIMAL TISSUES

Mammals	Vitamin A is stored mainly in the liver Other sites of concentration are the kidney lungs, adrenals and retina The blood plasma contains a lower concentration of vitamin which tends to remain at a constant level characteristic of the species and individual The fat deposits also contain small amounts of vitamin The location of vitamin A in histological sections can be demonstrated by fluorescence microscopy In the liver the strong fluorescence of the Kupffer cells indicates a high concentration of the vitamin Centrifugation of liver homogenates however, has failed to give clear evidence of the concentration of the vitamin in any particular structure Thus the 'soluble fraction" contained more vitamin than the nuclei or mitochondria
Birds	The liver is again the main site of storage In certain sea birds the stomach contains oil which is also rich in the vitamin
Fish	In some fishes large amounts of vitamin sometimes even exceeding those in the liver, can be found in the intestinal walls In eels large amounts of the vitamin are located in the body fat
Shrimps	In some species of shrimps almost the entire vitamin A contents of the body are concentrated in the eyes

Homarus americanus has vitamin A three times more concentrated in its eyes than in its hepatopancreas, but the greater weight of the hepatopancreas doubtless makes it the most important contributor to the total vitamin contents of the body.

Carotenoids The selective absorption of carotenoids into the bodies of animals has been discussed in Chap 13 Here we may note

may vary considerably in the depth of its yellow colour. There are certain specialised sites, moreover, in which carotenoids are concentrated, at least in those species having yellow fat Thus the "*copora lutea*" of the ovary get their name from the yellow material, rich in lipoid, which is formed in the follicles from which the ova have been erupted Escher [21] proved that the

are not observed in the ovaries of animals which have white body fat Van den Bergh, Muller and Broekmeyer [23] suggested that the *corpora lutea* of pigs might better be called *corpora alba*

The adrenals have already been mentioned as a site for the concentration of vitamin A In animals with yellow fat they may also contain considerable amounts of carotenoids Thus Bailly and Netter [24] isolated 0 3 g of β-carotene from 30 kg of bovine suprarenal glands The pigment was concentrated in the cortex and was virtually absent from the medulla [25] Carotene was also isolated from testes, the pituitary contained a trace but none could be detected in the thymus and thyroid glands [26]

Cellular Distribution of Vitamin A

Fluorescence microscopy Experience with normal human kidneys, as we have seen above, suggests that vitamin A may sometimes be present in the tissues without revealing its presence by fluorescence It is instructive, however, to study those intracellular sites in which the presence of the vitamin can be demonstrated, even if we may sometimes fail to perceive other sites which may also contain it By comparing the fluorescence of tissue sections from deficient animals with others from animals which have been dosed with the vitamin much useful information has been gained Fluorescence microphotographs of rat's liver and suprarenals taken by Popper and Greenberg [7] are shown in Plate 6 (page 217)

References p 219

In the livers of normally nourished animals these workers found that vitamin A was distributed in several localities Thus the livers cells contained small fluorescent granules which appeared to mark their boundary with the sinusoids The cytoplasm also fluoresced but less brightly In the Kupffer cells strong fluorescence was seen to originate mainly from small lipoid droplets situated close to the nuclei In deficiency of vitamin A the charac teristic fluorescence was absent from all these sites In animals which had been given massive doses the fluorescence was intensified particularly in the Kupffer cells We shall discuss later (Chap 27) evidence that the Kupffer cells are abnormal in their structure and function in deficiency of the vitamin

Vitamin A was also seen in adrenals where it appeared in the epithelial cells of the fascicular layer Normal rat kidneys in contrast to the finding for humans showed fluorescence in both the cortex and medulla Fine granules fluoresced in the interstitium between the cortical tubules and the vitamin also appeared to be present in the endothelial cells of the capillaries Normal lung fluoresced in the alveolar septums apparently in the capillary epithe lium and interstitial cells In the ovaries fluorescence was seen in the inter

escence suggestive of vitamin A

In the eye fluorescence was seen in the rod and cone layer of the retina and in the pigment layer in the form of strongly fluorescent fine droplets Al though in most other sites fluorescence was absent if the rat had been made deficient in vitamin A the fluorescence in the eye persisted in all stages of deficiency This remarkable finding was in keeping with the known persist ence in the eye of rhodopsin and of vitamin A as estimated by the antimony trichloride reaction after other parts of the body have become depleted

In the gastrointestinal tract of normal rats which had not been dosed recently with vitamin A fluorescence was seen in the fat cells in common with those in the other parts of the body When a heavy dose of vitamin A had recently been given however bright fluorescence was clearly seen in the lumen of the stomach and intestine In the duodenal and upper part of the jejunum fluorescence appeared in the walls where the vitamin was presum ably being absorbed Thin streaks of fluorescence at the edges of the epi thelial cells of the villi indicated the passage of the vitamin from the lumen to the lamina propia Here the fluorescence was shown by granules carried in the cells or by thread like streams in the lacteals In the larger lacteals the fluorescence could be traced from the submucosa to the sub-serous layer

Fluorescence suggestive of vitamin A was sometimes seen in the pars intermedia of the pituitary The brain spleen testes thyroid gland lymph nodes oviducts tongue skin and muscle did not reveal the typical fading

fluorescence of vitamin A As a matter of general interest however permanent fluorescence of different colours was seen in various sites Dim white fluorescence for example was sometimes observed in the stroma of the intestinal villi and brown fluorescence in the ovaries

Fractionation of liver cells Other evidence on the distribution of vitamin A in the tissues has been obtained by chemical methods Thus Goerner[27] reported the presence of vitamin A in the mitochondria of the liver cells of rabbits The mitochondria were separated by the fractional centrifugation at different speeds of an aqueous suspension of the finely divided tissues For the estimation of the vitamin the reaction of Rosenthal and Erdelyi[28] was used which has been criticised on the grounds that it may also be given by sterols More recently the use of isotonic sucrose for suspending the tissue[29] has greatly facilitated fractionation

Using this method presumably in conjunction with the antimony trichloride method Collins[30] found that a single rat liver contained 23 i u of vitamin A in the nuclear fraction 137 i u in the mitochondria 2 7 i u in the microsomes and 520 i u in the soluble fraction By similar methods Powell and Krause[31] separated finely divided and strained rat liver into nuclei mitochondria and a fraction X consisting of the remaining cytoplasm In rats which had not been specially dosed with vitamin A the total homogenate averaged 623 i u per g the nuclei 97 i u mitochondria 117 i u and fraction X 386 i u The percentage contributions by the three fractions were thus 15 19 4 and 64 6 respectively which agrees with the finding of Collins that most of the vitamin is in the soluble fraction In animals which had been heavily dosed with vitamin A the distribution of the vitamin was much the same except for a small increase in the nuclear fraction at the expense of fraction X

STATE OF COMBINATION OF VITAMIN IN VARIOUS SITES

Esterification Except in the blood and probably also in the retina vitamin A is mainly present in esterified form As early as 1928 Bacharach and Lester Smith[32] sagaciously inferred that the vitamin could form esters from their knowledge of the commercial Zucker process in which alcohol was used to extract vitamin D from cod liver oil Vitamin A could not be extracted from the oil in this way although its concentrates were known to be freely miscible with alcohol Saponification therefore induced some change in the vitamin which was correctly interpreted even before the chemical nature of the vitamin had been fully established Reti[33] extended the conclusion that vitamin A is present as esters in the liver to apply generally to various fish birds and mammals

We have already discussed the changes between the free vitamin A and

References p 219

its esters during absorption from the intestines (Chap 18) Further information on the differences between vitamin A as ingested and as stored in the liver has been provided by Gray, Hickman and Brown [34] From a study of the distillation curves of rats liver oils they concluded that vitamin A which had been given as massive doses of the free alcohol or caproate was converted to esters by the selective utilisation of the fatty acids native to the rat Later Gray and Cawley [35] made similar studies on rats which had been given smaller doses of fish liver oils or concentrates over a long period The vitamin now appeared to be stored in the rat's liver mainly as a single ester probably the palmitate

Although esterified vitamin A always predominates in liver oils traces of the free alcohol can usually be found At one time the alcohol was considered to be due to slight hydrolysis in the oil caused by staling, but Mead [36] found small amounts of free alcohol in oils which were specially prepared under conditions which minimised hydrolysis Glover, Goodwin and Morton [37] suggested that vitamin A is present in the liver cells as the free alcohol, probably combined with protein and in the Kupffer cells in the form of esters This interesting suggestion lacks proof, but there is at least some evidence that vitamin A alcohol and esters differ in their distribution in the various fractions of liver homogenates Thus Krinsky and Ganguly [38] found that the ratio of esters to free alcohol was 47–261 to 1 in the upper ' creamy centrifugal layer but only 3–35 to 1 in the supernatant fraction and 5–27 to 1 in the microsomal fraction The last two fractions contained 36% of the total alcohol recovered and less than 9% of the esters

Hydrolysis The presence of an enzyme capable of hydrolysing vitamin A esters has been inferred from experiments *in vitro* on blood liver and other tissues Krause and Alberghini [39] observed at least partial hydrolysis when rat s blood rich in esterified vitamin A was incubated at 37° C for 3 hours The factor responsible appeared to be present in the plasma rather than in the cells The activity of rat's liver homogenate was studied in some detail by McGugan and Laughland [40] An enzyme was demonstrated which could hydrolysed limited amounts of vitamin A acetate It was thermolabile inactivated by alcohol and had an optimum pH of about 8 5 The amount of ester hydrolysed was proportional to the amount of homogenate used in each test In contrast to the experience of Krause and Alberghini with blood the activity was associated with the cell fragments and a cell free dialysate had only slight activity Lesser activity than in liver was found in blood and intestinal mucosa, and even less in heart, spleen and kidney

According to Krause and Powell [41] rat liver homegenate or any of its fractions can hydrolyse vitamin A acetate but not the palmitate On a wet

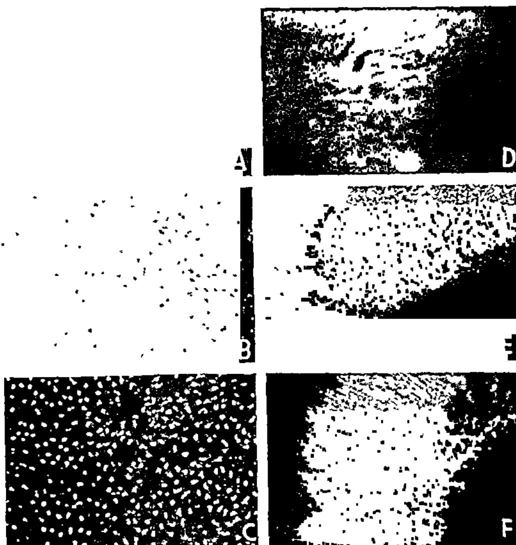


Plate 6 The distribution of vitamin A in the tissues of the rat as studied by fluorescence microscopy The photomicrographs on the left are of liver and those on the right of suprarenal A Liver from a deficient rat shows virtually no fluorescence B Liver from a normal rat shows fluorescence in the Kupffer cells and in places in fine droplets at the edge of the liver cells C Liver from a hypervitaminotic rat shows strong fluorescence in the Kupffer cells which appear to be proliferated Moderate fluorescence is also shown by the small lipoid droplets within the liver cells D Suprarenal of a deficient rat E Suprarenal of a normal rat showing moderate fluorescence in the lipoid droplets in the fascicular layer F Suprarenal of hypervitaminotic rat with strong fluorescence in the fascicular layer but still not in the outer glomerular layer (By courtesy of Dr Hans Popper and of Archives of Pathology)

basis nuclei, mitochondria and "fraction X" were all equally effective in hydrolysing the acetate, but on a basis of nitrogen content fraction X' was rather less active than the other two fractions The reverse process of esterifying vitamin A could be effected by homogenates of intestine and kidney but not by liver homogenates A different story, however, was told by Ganguly and Deuel [42] In their experience the ability to hydrolyse vitamin A acetate was concentrated in the microsomal fraction, with the nuclear mitochondrial and supernatant fractions all inactive

Combination with protein Although the evidence falls short of complete proof it seems highly probable that carotene and vitamin A alcohol are held in blood plasma by combination with protein Dzialoszynski Mystkowski and Stewart [43] studied the response of both substances to extraction from human plasma With ether as solvent the ease of extraction was increased by lowering the pH or by the addition of alcohol Precipitation of the globulins by half saturation of the plasma with ammonium sulphate did not carry down carotene or vitamin A, but they accompanied the albumins which were precipitated by $^3/_4$ or complete saturation It was concluded therefore that the vitamin and provitamin are carried in the blood attached to albumin In similar studies on the plasma proteins of various species by Ganguly *et al* [44] carotene, vitamin A esters and vitamin A alcohol were found to be present in different fractions Vitamin A esters were associated with the least soluble protein fractions, but vitamin A alcohol in agreement with Dzialoszynski and colleagues, adhered to the more soluble fractions The work of Krinsky and Ganguly, reviewed above, suggests that in the liver cell free vitamin A is associated with protein, and presumably esterified vitamin A with fat

The association between vitamin A and protein in the retina will be discussed in Chap 22

TABLE 23

POINTS OF INTEREST IN REGARD TO THE ESTERIFICATION OF VITAMIN A

Liver and tissues	Contain vitamin A mainly in esterified form
Blood plasma	Except for vitamin A newly absorbed from the intestines most of the vitamin is present as the free alcohol loosely attached to protein (albumin ?)
Hydrolytic enzymes	The presence of enzyme systems capable of hydrolysing vitamin A esters has been demonstrated in blood plasma and in homogenised liver Attempts to locate the enzyme in various centrifugal fractions of liver have so far given inconclusive results

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CHAPTER 20

The Mobilisation of Reserves of Vitamin A from the Liver, and the Rate of their Expenditure in Relation to the Physiological Requirements of the Body

In the preceding chapters we have followed the absorption of vitamin A from the intestines, and have studied its distribution in the liver and other parts of the body We must now reverse the picture, and discuss such evidence as is available on the mobilisation of stores of vitamin from the liver back into the blood stream, and their expenditure in the course of metabolism

We have already mentioned that a delicate mechanism adjusts the level of vitamin A in the blood so that it remains constant in spite of large variations in the stores held by the liver It will be convenient to deal first with short-term experiments on the effect of various treatments on influencing the balance of this mechanism, with the result that the level of vitamin in the blood is increased Any evidence of corresponding decreases in the liver reserves in these short-term experiments can be discussed at the same time Secondly we must deal with investigation of a rather different type, in which the rate of expenditure of the liver reserves has been studied, usually over a prolonged period, without reference to the level in the blood As a preliminary to understanding investigations of the second type it will be helpful to review evidence on the physiological requirements of certain species for the vitamin

The Mobilisation of Vitamin A into the Blood Stream

Liver operation An early clue on the mobilisation of vitamin A was found in 1935 by Drummond and McWalter[1] in the course of attempts to prove that carotene could be converted to vitamin A in liver When one lobe of the liver was removed from rats the concentration of vitamin A in the remaining lobes, when examined shortly afterwards, was much lower than in the first lobe It appeared, therefore, that the operation caused the disappearance of vitamin A from the portions of liver which had not been removed The same observation was made on rabbits by Young and Wald[2], who made the additional finding that after the operation the level of vitamin A in the blood reached 5–7 5 times its former level

Nervous stimulation

Another early observation, also in 1935, was made by Chevallier, Malméjac and Choron [3] who studied the effect of nervous stimulation on the mobilisation of vitamin A in dogs They found that after stimulation of either the pneumogastric or the splanchnic nerve the level of vitamin in the blood was nearly doubled Young and Wald [2] obtained the same result by stimulating the splanchnic nerve, but not by stimulating the cervical sympathetics

Role of the adrenals

The French workers [4] recalled that Bailly and Netter [5] had reported that in bovines the adrenal glands are very rich in carotene Acting on this clue they designed experiments intended to decide whether nervous stimulation might exert its action in mobilising vitamin A through the medium of the adrenals For this purpose pairs of dogs were anastomosed so that the blood from the suprarenal vein of the one passed into the jugular vein of the other When the splanchnic nerve of the first dog was stimulated the level of vitamin A in its own blood was not affected, but an increase was observed in the second dog The same effect was observed even when the medulla had been removed from the adrenal of the first dog The vitamin could therefore be mobilised by the secretions of the adrenal cortex, which do not include adrenaline

Adrenaline

Although Chevallier s work pointed to the importance of the adrenal cortex, Wald and his colleagues found that injections of adrenaline, a product of the medulla, were effective in raising vitamin A in the blood of rabbits Thus intravenous injections of about 1 mg of adrenaline into five rabbits caused the average vitamin A contents of the whole blood to increase from 80 i.u per 100 ml before the injections to 160 i u thirty minutes after the injections These results suggested that vitamin A might be controlled by the same sympathetic adrenal system which is responsible for the mobilisation of sugar, and of certain plasma proteins, from the liver into the blood stream

Thiele and Guzinski [6] confirmed that adrenaline increased vitamin A in the blood in experiments on human subjects The values found after the injection of the hormone were invariably some 25% above the resting level Goodwin and Wilson [7], however, failed to repeat Wald's observations on rabbits No consistent differences were found, with slight increases in some animals and decreases in others In rats the average blood vitamin A level was increased by only about 4%, which can hardly have been statistically significant In human subjects who were given intramuscular injections of adrenaline Hillman [8] observed increases in some cases but decreases in others The average for a group of 22 control subjects was about the same before and after the injections No correlation was found between the increases in vitamin A and the mobilisation of sugar into the blood For the

References p 235

present, therefore, the possible effect of adrenaline on vitamin A metabolism must remain open to question

Adrenal cortical hormones Chevallier's early indication that the adrenal cortex is concerned with the mobilisation of vitamin, irrespective of the doubtful action of the medulla, has recently been strengthened by observations by Clark and Colburn[9] In one of their interesting experiments young male rats which had total vitamin A reserves of the order of 1671 u were given *ad lib* a diet deficient in vitamin A Some of the animals were given subcutaneous injections of 3 mg of cortisone daily for 13 days which was sufficient to inhibit growth completely Other animals received no hormone The total liver reserves were measured in pairs of rats from both groups killed at intervals, and the results are given in Table 24

TABLE 24

THE EFFECT OF CORTISONE IN DECREASING VITAMIN A IN THE LIVERS OF RATS (CLARK AND COLBURN)

No of days dosing	*Av body wt*		*Av liver wt*		*Total vit A in liver i u*	
	Cortisone	*Control*	*Cortisone*	*Control*	*Cortisone*	*Control*
1	94	104	6 3	5 8	181	142
3	94	118	6 1	6 7	152	191
5	91	117	5 9	6 3	89	155
9	96	140	5 8	8 4	59	188
11	85	130	5 3	7 4	39	158
13	85	145	6 0	7 6	36	158

It will be seen that there was no measurable depletion of vitamin A in the control animals, which was not surprising in a short term experiment In the animals injected with cortisone, however, the total reserves fell to about 20% of their original level The influence of the adrenal cortex on the level of vitamin A in the blood stream has been studied by Jackson and his colleagues[10] In patients with rheumatic fever vitamin A was increased by injections of pituitary corticotrophin (ACTH) It might be argued, of course, that the effect on the vitamin was secondary to the general alleviation of symptoms caused by the hormone Increases were observed however, in both the acute and subacute phases of the disease

But while the total cortical secretion appears to mobilise vitamin A into the blood, it is clear that this action is not exerted by all members of the complex when administered singly Bodansky and Markardt[11] have found in experiments with rats that Reichstein's compound L, or 3 β acetoxy 17α-hydroxyallopregnan 20 one, tends to reduce the level of vitamin A in the

blood Moreover when compound L is administered in conjunction with low supplements of vitamin A it increases storage in the liver and decreases storage in the kidney Its action therefore, seems to resemble that of oestradiol (see below)

The sex hormones The extensive evidence on the effect of sex on vitamin A metabolism will be discussed fully in Chapter 35, and for the present a summary of the main findings will suffice In the blood it has been found, in numerous investigations on humans and on rats, that vitamin A tends to be slightly higher in males than in females This difference cannot be demonstrated between individuals, but is found consistently between the averages for groups of each sex In humans the superiority in carotene is in the opposite direction to that found for vitamin A, with slightly higher averages for women than for men

In the liver higher reserves of vitamin are accumulated in female rats than in males receiving the same diet In the kidney males tend to have the higher storage, and for some obscure reason the highest concentrations are found at levels of dosing which barely allow storage in the liver

The general picture, therefore, indicates a greater retentive power of the liver in the female than in the male In the reverse direction, of course we may consider that in the male the mechanism is more active for mobilising the vitamin into the blood, and thence into the kidneys

In the rat sex overlaps to some extent with growth in its effect on vitamin A metabolism, but it seems clear that sex has at least a residual influence which does not depend on growth Thus vitamin A remains low in the kidneys of females even during rapid growth In castrated male rats oestradiol both checks growth, and lowers vitamin A in the kidneys to the female level Restriction of the food intake to produce the same retardation in growth as caused by oestradiol certainly lowers vitamin A in the kidneys, but the level remains substantially above that typical for females

If oestradiol strengthens the power of the liver to absorb vitamin A it might be expected that testosterone would act in the opposite direction Attempts to substantiate this suggestion, however, have been unsuccessful Little difference could be found in vitamin A metabolism between entire and castrated male rats, or between castrated rats with or without injections of testosterone Attempts to produce the male characteristics of vitamin A metabolism in female rats, by ovariectomy and injections of testosterone, have also been unsuccessful

Alcohol Taking a long step from the endocrine systems we must next turn to alcohol as a factor capable of mobilising vitamin A into the blood stream In 1940 Clausen *et al*[11] gave 60 ml of ethyl alcohol diluted with water, to eight fasting dogs In specimens of blood which were collected be-

References p 235

fore and after dosing increases of 50 to 300 per cent followed the adminis tration of the alcohol Sometimes the maximum value was not reached until 48 hours after dosing In two dogs with bile fistulas no increase in vitamin A was found in the lymph It appeared, therefore, that vitamin A was mobilised from the liver

Later a more detailed paper [13] described further studies Ethyl alcohol increased the blood vitamin A both when given orally or by intravenous or intraperitoneal injection In a single dog daily injections of alcohol in the morning consistently produced increases in specimens of blood collected in the afternoon Intravenous injections of methyl, propyl or *iso*propyl alcohols diluted with saline, not only raised the blood vitamin A, but also made the animals very intoxicated Vitamin A estimations on specimens of liver, ob tained by biopsy, suggested that the greatest increases in blood levels occurred in those dogs which had the highest reserves Both before and after dosing with alcohol most of the blood vitamin A was present in the esterified form

The effect of ethyl alcohol in increasing the level of vitamin A in the blood was confirmed in calves and goats by Crowley and Allen [14] The levels in both animals were usually about doubled within 4 hours after dosing with about 50 ml of alcohol per 100 lbs of body weight In contrast to these findings however, attempts to increase vitamin A in human blood by giving alcohol have been unsuccessful Yudkin [15] found no consistent changes in the blood level of adult volunteers who had been dosed with 20 ml of alcohol in the form of sherry wine even although dark adaptation was always slightly improved Hume and Krebs [16], who gave 80–100 ml of alcohol agreed in find ing no alteration in the level of vitamin A in the blood The efficiency of dark adaptation deteriorated in parallel with the effect of alcohol in depressing the mental processes

Other factors The observations on the effect of alcohol in raising vitamin A in the blood recall an early report by Chevallier and Choron [17] that ether anaesthesia has this effect in rats and guinea pigs This point seems worth further investigation

According to Solyanikova [18] the blood level of vitamin A in rats responds to large doses of vitamin D At first vitamin A is increased in the blood and decreased in the liver and adrenals More prolonged dosing with vitamin D, however causes vitamin A to decrease in the blood and liver, but to increase in the adrenals Eventually all sites become lower in vitamin A than in con trol animals

In calves Thomas Jacobson and Moore [19] have found that the level of vitamin A in the blood is influenced by dietary factors other than the amount of vitamin and provitamin which are available Restriction of animals

possessing liver reserves of vitamin A to certain diets from which the vitamin and provitamins are absent may actually cause the level of vitamin in the plasma to increase The mobilisation of vitamin into the blood is favoured by diets which contain skimmed milk and which are low in fat

Physical exercise was found by James and ElGindi[20] to increase the level of vitamin A by margins of 20 to 106% in the blood of 11 out of 12 college athletes The one athlete to show a fall in vitamin A was in good condition whereas the athlete showing the largest increase was in poor condition The blood carotene was decreased after exercise in 9 of the 12 subjects According to an early report by Ratchevsky[21] great physical efforts and also alcohol inanition and moral affections all caused reduction in the carotene level in the blood

In contrast to exercise hyperthermia whether induced artificially or by disease decreases vitamin A in the blood This effect which is very well authenticated will be mentioned again in Chapter 32 For the present we may remember that exercise tends to increase the body temperature We may wonder therefore why exercise does not also decrease rather than increase the level of vitamin A in the blood Presumably the answer is to be found in the difference between the respiratory circulatory and endocrine changes during exercise and during fever or artificially induced pyrexia

PHYSIOLOGICAL REQUIREMENTS FOR VITAMIN A

As a preliminary to discussing studies on the rate of expenditure of the body s reserves of vitamin A during dietary deprivation of the vitamin it will be helpful to summarise evidence on the physiological requirements of various animals for the vitamin In interpreting observations on the rate of fall in the reserves of vitamin we may then be able to decide how much of the vitamin was being used for physiological purposes and how much is running to waste

Requirements of the rat The most extensive and fruitful studies on the expenditure of vitamin A have been made on rats It will be convenient therefore to deal in some detail with the requirements of this animal in this chapter

In the experience of most early workers[22] about 2 i u of vitamin A or carotene sufficed to restore growth in deficient rats It was realised however that much larger intakes were necessary to promote maximum growth or to allow storage of the vitamin in the liver Observations by Lewis *et al*[23] on the effect of graded doses of preformed vitamin A on the growth of deficient rats and on the levels of vitamin in livers and blood plasma after 6 weeks of dosing are summarised in Table 25 It will be seen that growth at the maximum level was given by doses of about 25 i u daily Doses of about 50 i u

References p 235

were necessary, however, to bring the level of vitamin in the blood near its maximum, or to cause the appearance of appreciable stores of vitamin in the liver The sex of the rats was not stated

TABLE 25

VITAMIN A REQUIREMENTS OF YOUNG RATS (LEWIS *et al*)

Daily dose i u for 6 weeks	*Av body wt*		*Av vitamin A in*	
	Initial	*Final*	*Blood plasma i u /100 ml*	*Liver i u /g*
0	45	98	0	0
1	44	119	7	0
2	40	140	14	0
10	40	159	35	0
25	41	172	69	3
50	41	172	100	34
100	42	173	112	113
1000	43	177	110	1270

Sherman and his colleagues [24, 25, 26] gave groups of rats allowances of 3, 6 and 12 i u of preformed vitamin A per g of dry diet, which may be calculated as equivalent to daily doses of 45, 90 and 180 i u for adult rats eating 15 g of food daily The total length of life, and also the period of sexual activity, were increased with the magnitude of the dose In further experiments however, raising the allowance to 24 i u per g of food caused no further improvement, and performances were perhaps not as good as with only 12 i u For the accumulation of more than traces of vitamin A in the liver the 6 i u allowance was necessary, and it was noticed that the reserves accumulated by females were about double those accumulated by males

Fraps [27] confirmed that the longevity of rats was influenced by the level of their vitamin A allowance In his investigation, however, the doses given were much lower than those chosen by Sherman Thus groups of young male or female rats were reared on diets which contained either 0 1 or 0 2 μg of purified carotene per g of diet, or its equivalent in other sources of carotene or preformed vitamin A These allowances correspond to only about 2 5 and 5 0 i u daily for rats eating 15 g of food daily In Table 26 averages for longevity and maximum body weight have been calculated for each level of dosing irrespective of the form in which the vitamin was given It will be seen that the body weights attained at the higher level of dosing were somewhat greater than with the lower level of dosing A much larger difference, [illegible] dosing however, was found in longevity, with [illegible] surviving about twice as long as those [illegible]

vitamin A reserves were made, but at such low levels of dosing the livers presumably contained only small traces of vitamin

TABLE 26

VITAMIN A REQUIREMENTS OF THE RAT (FRAPS)

Dose carotene µg per g of food	*Average length of life days*			*Average maximum body weight g*		
	Males	*Females*	*Average*	*Males*	*Females*	*Average*
0 1	263	225	244	282	188	235
0 2	526	548	537	359	224	292
0 2*	454	396	425	293	196	245

* Duplicate experiment

The results of similar experiments by Paul and Paul [28] are summarised in Table 27. With daily doses of 1, 2, 4 and 20 i u of preformed vitamin A per 100 g of body weight the maximum weights attained and times of survival were progressively increased Between the 20 and 4 i u levels of dosing, however, the differences were much smaller than between 4 and 2 and 1 i u In contrast the disparity between the 20 and 4 i u in preventing abnormalities in the eyes or teeth was even greater than between the other levels of dosing.

TABLE 27

VITAMIN A REQUIREMENTS OF THE RAT (PAUL AND PAUL)

Daily dose i u per 100 g rat	*Survival time days*	*Maximum body wt g*	*Percentage eye abnormalities*	*Dental abnormalities (maximum 4)*
1	80	100	96	Died
2	234	220	74	3 3
4	521	320	40	2 05
20	649	360	5	0 08

From a survey of the above evidence, combined with the author's own experience, it appears that a minimum dose of 1–2 i u of vitamin A or carotene is generally sufficient to restore growth in a young rat which has been deprived of vitamin A At this rate of dosing growth may continue until about 70% of the normal maximum body weight is revealed, but death may be expected within 8 or 9 months Signs of partial deficiency will be seen in depigmentation of the incisor teeth, and in poor general condition Increasing the dose to 4 i u may about double the time of survival, but further improvements in longevity will result from increases up to 50–100 i u daily. In

References p 235

order to allow the storage of vitamin A in the liver, doses of 20–40 i u of preformed vitamin A daily are necessary, with somewhat higher doses for males than for females To allow experimental rats to accumulate reserves equal to those of wild animals doses of at least 100 i u daily seem necessary These conclusions may be summarised in simplified form, as follows

Dose necessary for	*i u per rat daily*
Growth restoration	2
Intermediate longevity	4
Detectable storage of vitamin A	30
Full longevity	100
Natural' storage	100

These figures point to the somewhat unexpected conclusion that more vitamin A is required to prolong life in the adult rat than to support growth in the young rat Evidence in favour of this view, based mainly on the condition of the teeth of rats after varying periods of deficiency and at graded levels of dosing, was presented in 1939 by Irving and Richards[29]

Other animals The requirements of man will be discussed in Chapter 30 and those of farm animals in Chapter 34 At this juncture, however, it may be helpful to touch on the question of the relationship between vitamin A requirements and body weight

As a rough estimate we may consider that an average human adult, weighing 70 kg, has a daily intake of vitamin A, in all forms, corresponding to 2500 i u of the preformed vitamin This allows the maintenance of normal health and the accumulation of substantial reserves of vitamin A in the liver, perhaps 350 i u per g

For a rat weighing 200 g the corresponding intake of vitamin calculated directly upon body weight, would be only about 7 i u This would be well below the allowance necessary for maximum longevity, or for the storage of more than traces of vitamin in the liver It therefore appears that the requirements of the two species cannot be compared on the simple basis of body weight

By correlating the vitamin A requirements with the relative food intakes however, a much more reasonable relationship may be established For the human we may take the food intake as 3000 calories and for the rat as 75 calories A human intake of 2500 i u would therefore correspond to 63 i u in the rat, which would be adequate for a long life and for the accumulation of substantial stores of vitamin

The Expenditure of the Liver Reserves of Vitamin A

We are now in a position to discuss the degree of efficiency with which the vitamin A reserves of the liver can be used to supply the needs of the body when the diet is defective in the vitamin There can be no doubt, of course, that the presence of a reserve does allow the animal to survive during deprivation, and that the time of survival roughly depends on the magnitude of the reserve It is more questionable, however whether the animal always employs its reserves, particularly when they are high, to the best possible advantage It will be recalled that Popper and Brenner[30] have suggested that after heavy dosing vitamin A may be destroyed by the Kupffer cells of the liver, or may be retained by them temporarily and then released into the rest of the body as a means of disposal It is also possible that the vitamin, whether absorbed by the Kupffer cells or not, may be lost down metabolic side paths when large quantities have to be held in the body for long periods.

Rate of fall of liver reserves The impressive calculation that rats given vitamin to las

Moore[31] to a

Adult females were first given vitamin A until their livers appeared incapable of holding any more The concentrations reached were usually about 10,000 i u per g of liver The animals were then given a commercial diet deficient in vitamin A, and groups were killed at chosen intervals

The mean total reserves were as follows

0 weeks of deficiency	100,000 i u
4 ,, ,, ,,	16,000 ,,
8 ,, ,, ,,	15,000 ,,
12 ,, ,, ,,	2,400 ,,
24 ,, ,, ,,	3,000 ,,

At the commencement of deficiency, therefore, there was a rapid loss of vitamin, which was quite beyond the range of physiological requirements Thus during the first 4 weeks the loss amounted to 300 i u per g of liver daily, or 3000 i u daily for the whole liver For the next 8 weeks of deficiency the loss was about 24 i.u. per g of liver, and during the last 4 weeks there was no further loss It seems probable that the differences found between 4 and 8 weeks of deficiency and between 12 and 24 weeks would have followed a smoother curve if larger numbers of animals had been used. Clearly, however, a very rapid loss of vitamin occurred during the first weeks of deficiency with much slower losses during later weeks

References p 235

Later another experiment of the same nature was made [32] It differed, however, in the use of young growing rats, in the choice of a basal diet of the conventional type, and in limiting the preliminary storage of vitamin A to about the level found in wild rats. The results were as follows

Days of deficiency	*Condition of rats*	*Total reserves of vitamin A i u*	*Daily loss of vitamin, i u*
0	Growing	2300	—
42	"	540	42
84	"	280	6
175	Growing stopped	0	3

In parallel experiments rats, which had been allowed to accumulate total reserves of only about 25 i u before restriction to the deficient diet, stopped growing after about 35 days, by which time their reserves were completely exhausted There can be no doubt, therefore, that high vitamin A reserves, even if used up at an excessive rate, can nevertheless greatly prolong the survival of animals during dietary deficiency In the experiment just described survival was extended for about 5 months During the first weeks of depletion the rate of expenditure was of the order demanded for optimum growth and longevity, while during later weeks the expenditure fell towards the level necessary to restore temporary growth in young rats acutely deficient in the vitamin

Attention must be drawn, however, to certain interesting exceptions to the general rule that survival times can be predicted from the magnitude of the vitamin A reserves Dann [33] and also Baumann, Rising and Steenbock [34] have shown that when young rats are given a deficient diet they may continue to grow rapidly for several weeks after the livers of their companions have been shown to be quite devoid of the vitamin As the reverse of this picture Guilbert and his colleagues [35] [36] reported that when cattle and poultry were subjected to prolonged vitamin A deficiency clinical and pathological signs of vitamin A deficiency became evident while the liver still contained traces of vitamin A, as demonstrated by the antimony trichloride method

Expenditure in relation to the magnitude of the liver reserves. From the experiments of Davies and Moore it might well have been concluded that the loss of vitamin A at any time during dietary deprivation is proportional to the magnitude of the reserves remaining at that time In 1946 this theory was developed by Hickman [37] and was supported by observations by Frey and Jensen [38] on the loss of vitamin A

reserves by calves When the animals were given a fattening diet deficient in the vitamin the following results were obtained

Days on diet	*Liver reserves of vitamin A i u /g*	*Percentage decrease*
0	171	—
40	81	53
80	37	54
120	17	54
160	7	59

It seems probable that the loss of reserves often follows this type of curve Unpublished evidence by the author however has suggested that high reserves may be held more tenaciously on some occasions than on others Thus in one experiment the average total reserve of six rats after 9 months of deprivation was still 37 000 i u as compared with 61 000 i u found in animals killed before deprivation

The influence of vitamin E It is often difficult in spite of the progress of research to recognise the true cause of the wide differences in vitamin A reserves which are often observed between individuals which have received the same dietary treatment Variations in the rates of expenditure between individuals or between experiments may also be difficult to explain It is clear however that the vitamin E status can have dramatic effects both in storage and on expenditure The effect of toco pherol on the storage of vitamin A and on the response of rats to small doses of vitamin A or carotene has been discussed in Chapters 16 and 18 Its effect on the expenditure of stored vitamin A was studied by Davies and Moore [39] Rats which had total reserves averaging 20 000 i u were restricted to a diet deficient in both vitamins A and E which was supplemented for some of the animals with adequate doses of *dl α* tocopheryl acetate The results were as follows

Days of deficiency	*Average total vitamin A reserve i u* + *Vitamin E*	— *Vitamin E*
0	20 000	20 000
28	25 000	16 500
91	13 000	4 700
222	5 800	9

It will be seen that the vitamin A reserves decreased with the duration of deficiency in both groups The depletion was more rapid however in the

References p 235

animals deprived of vitamin E than in those with supplements Thus after about 7 months the rats without vitamin E were virtually deficient in vitamin A, while those dosed with tocopherol had all substantial reserves (3700–3500), presumably sufficient to last for many more months It is possible, therefore, for vitamin A deficiency to occur as a secondary effect of vitamin E deficiency

A striking illustration of the necessity of vitamin E for the storage of vitamin A has been given by Dam, Prange and Søndergaard [40]. When rats were given a diet containing vitamin A in the form of 10% of cod liver oil, but no vitamin E, their vitamin E reserves after 5 weeks averaged 1864 i u per g After 14 weeks, still with cod-liver oil in the diet, the reserves of similar animals had fallen to 536 i u per g In rats dosed with *dl α* tocopheryl acetate the reserves after 14 weeks had risen to 3738 i u per g A similar rapid fall in the vitamin A reserves, in spite of liberal dietary supplies was also observed by the author in a batch of vitamin E deficient rats which were dosed with about 5000 i u of vitamin A weekly as halibut liver oil

Growth and basal metabolic rate In view of the claim that the requirement for vitamin A increases with age it might seem questionable whether the process of growing makes any special demand on vitamin A metabolism In rats of the same age, however, Johnson and Baumann [41] have found that the expenditure of vitamin A is significantly affected by the rate of growth Thus slowly growing rats which were both deprived of vitamin A and restricted in growth by inadequacy of calories, tryptophan or aneurin, were found to use up their vitamin A reserves less rapidly than companions which were only deprived of vitamin A The expenditure of vitamin A also depended on the basal metabolic rate, being increased by thyroid and decreased by thiourea or thiouracil The effect of a reduced calorie intake in retarding the expenditure of vitamin A however, outweighed the accelerating effect of thyroid when given at the same time (See also Chap 37) In rapidly growing rats but not in rats whose growth was restricted, vitamin A was transferred from the liver to the kidney

Carcinogenic agents Between 1938 and 1942 an interesting sequence of papers appeared on the effect of carcinogens on vitamin A metabolism Goerner [42, 43] studied the effect of injections of the carcinogenic agent, 1,2,5 6 dibenzanthracene on the vitamin A metabolism of rats and rabbits Vitamin A, as measured by the method of Rosenthal and Erdélyi, was rapidly lost both from the enlarged livers, taken as a whole, and from their mitochondria *p*-Aminoazobenzene had relatively little effect in accelerating the expenditure of vitamin A The closely allied 2 amino-5 azotoluene, however, caused enlargement of the liver, and eventually hepatic tumours when given orally to rats over a long period Vitamin A was

decreased in the enlarged livers When tumours had been formed no vitamin could be detected in the hepatoma but the vitamin was still present in the surrounding liver cells

Baumann and his colleagues[44, 45, 46] made similar observations on dibenzanthracene using the conventional antimony trichloride method for estimating the vitamin Methyl cholanthrene and benzpyrene two more potent carcinogens and 1 2 benzanthracene which is non carcinogenic all had the same effect in removing vitamin A from the liver but to a less degree than dibenzanthracene The loss of vitamin from the liver was accompanied by a gain in the kidneys Butter yellow and *p* dimethylaminoazobenzene which are carcinogenic and carbon black which is not had no effect on vitamin A *metabolism The effects in producing cancer and in affecting vita* min A metabolism did not therefore run parallel The livers of rats with spontaneous tumours moreover contained more vitamin A than was found in non tumourous controls Loss of vitamin A was therefore not a pre requisite of tumour formation Abels *et al*[47] suggested that dibenzanthra cene competes with vitamin A for some substance probably a protein by which either can be bound to the liver

Carcinogenic properties of a different type were studied by Beck and Peacock[48] in cooking fat which had been repeatedly heated to a high tem perature The production of gastric ulceration and papillomatosis as describ ed by Roffo[49] was apparently associated with decreased vitamin A reserves in the liver

Liver injury Damage to the liver by agents other than carcinogens may also cause loss of vitamin A but some types of injury do not affect the storage of the vitamin Thus we shall see later (Chapter 32) that cirrhosis of the liver is often associated with complete exhaustion of the reserves *On the other hand Lasch*[50] *has shown that in experimental phos* phorus poisoning in rats and guinea pigs the vitamin A reserves are not affected presumably because the Kupffer cells escape damage *Clayton and* Baumann[46] found that the rate of depletion of vitamin A from livers which were heavily infiltrated with fat was the same as from normal livers In mice a repeated infiltration and removal of fat from the liver produced by alter nating between diets deficient and adequate in choline did not succeed in washing out vitamin A

Other factors The rate of loss of vitamin A must also be influenced by certain phases of reproduction such as the transfer of vita min to the foetus colostrum and milk (Chapter 21) *These demands on the liver* may be heavy and equivalent to a high percentage of the daily requirement of the adult when not engaged in reproduction

References p 235

During disease, and particularly during certain types of disease heavy losses of vitamin A from the liver usually occur (Chapter 32)

The fate of vitamin A after mobilisation from the liver

It seems reasonable to presume that the large amounts of unchanged vitamin A which are included in colostrum and milk contain a contribution from the liver reserves Losses of unchanged vitamin A, which must eventually influence the magnitude of the liver reserves may also occur in human urine in certain diseases (Chapter 32)

It must be clearly realised, however, that most of the vitamin A which disappears from the liver, either during dietary deficiency or during illness cannot be traced into other parts of the body, or into its secretions or excreta, in unchanged form It must be concluded, therefore that the vitamin is converted [illegible] by abnormal [illegible] mated, or even [illegible] the oxidation product reported by Le Page and Pett [61] may be one of these products

TABLE 28

FACTORS WHICH INFLUENCE THE MOBILISATION OR EXPENDITURE OF VITAMIN A

	Vitamin A in			
	Blood plasma		*Liver*	
	Level raised	*Reduced*	*Raised*	*Reduced*
Partial hepatectomy	+			+
Nervous stimulation	+			
Adrenal cortex total secretion	+			
A C T H	+			+
Adrenaline	+(?)			
Compound L		+	+	
Oestradiol		+	+	
Alcohol	+			
Skimmed milk diet	+			
Exercise etc	+			
Vitamin E			+	
Carcinogenic agents etc				+

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CHAPTER °1

The Transfer of Vitamin A from Mother to Offspring

In mammals vitamin A is transferred from the mother to her offspring by passage through the placenta and by secretion in the colostrum and milk The amounts of vitamin passed through the placenta are usually very small in relation to the supplies available to the mother Thus the foetal liver and blood are much below the maternal in their vitamin levels The colostrum however is a surprisingly rich source Its ingestion by the newborn young therefore makes an important addition to the small reserves which are usually present at birth although it may do little towards closing the large

place
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min contents as the time after parturition increases But although the con centration *of vitamin in the milk is low when compared with the amounts*

In birds and other oviparous animals a much simpler picture is seen Vitamin A is supplied to the offspring by inclusion in the egg yolk Apart from receiving food which has been partially masticated by the mother the offspring must depend on this one source of supply until it is able to eat for itself

Investigations on all aspects of the transfer of vitamin A between gener ations have been extensive The species mainly studied have been rats humans and particularly bovines which have been the subject of a volu minous literature Useful information is also available on sheep pigs and birds

The Transfer of Vitamin A via the Placenta

Rat In 1932 this animal was used for the first of an important series of studies by the author s esteemed colleague the late Dr W J Dann [1]

References p 260

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CHAPTER 21

The Transfer of Vitamin A from Mother to Offspring

In mammals vitamin A is transferred from the mother to her offspring by passage through the placenta and by secretion in the colostrum and milk The amounts of vitamin passed through the placenta are usually very small

[illegible]

therefore makes an important addition to the small reserves which are usually present at birth although it may do little towards closing the large gap between the levels of mother and offspring As the colostrum gives place to milk the concentration of vitamin A falls rapidly and even after full lactation has been established there is usually a further slow fall in the vitamin contents as the time after parturition increases But although the concentration of vitamin in the milk is low when compared with the amounts

[illegible]

In birds and other oviparous animals a much simpler picture is seen Vitamin A is supplied to the offspring by inclusion in the egg yolk Apart from receiving food which has been partially masticated by the mother the offspring must depend on this one source of supply until it is able to eat for itself

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References p 260

It was at once evident that the amounts of vitamin A passed into the foetal liver were small Another obvious conclusion was that the liberality of the mother's vitamin A allowance, supplied as carotene, had remarkably little effect on the vitamin A transferred to the young Thus the livers of young rats usually contained about 5 i u of vitamin A at birth, irrespective of whether the maternal total reserves were 600 or 12 000 i u When the liver of the mother contained only 12 i u of vitamin A, however, no vitamin A at all could be detected in the foetuses In another paper [2] the limited transfer of vitamin A to the foetus, in spite of enormous maternal reserves, was further emphasised Thus in mothers which were given vitamin A concentrate the reserves reached 180 000 i u , but the foetuses still contained only 20 i u each By increasing the level of fat in the diet from 1% to 20% the amount of vitamin transferred to the foetus could be slightly increased, perhaps by about 50%

Early work by Baumann, Rising and Steenbock [3] confirmed that the heavy dosing of pregnant rats with vitamin A only slightly increases the reserves of their young Thus foetuses from mothers which had received only a stock diet each contained 4 i u , which was raised to no more than 12 i u by giving the mothers daily doses of 5000 i u of vitamin A during the last 5 days of pregnancy Using modern methods of estimation Henry *et al* [4] also obtained data which agreed well with Dann's findings When the mothers were supplied with enough vitamin A to permit growth but insufficient to allow storage in their own liver, the foetal livers contained only about 1 i u each But within the wide range of 30 to 20,000 i u for the maternal liver the foetal livers were always of the order 5–10 i u,

Human Reliable information on the placental transfer of vitamin A must obviously be much more difficult to obtain for humans than for experimental animals, whose young can be killed as required It would be possible, of course, to confine the collection of specimens to infants who had died at birth from some gynaecological accident, but who otherwise could be considered normal In practice, however, difficulties may arise in accumulating data from sufficient cases, within a reasonable time, unless the ideal requirements can be somewhat relaxed Thus livers may be examined from premature infants, or from infants affected by disease in the mother for a short time after birth We shall be forced, therefore, to review such evidence as is available on [illegible] without insisting that all the data [illegible] extent of placental transfer

Most early workers, including Wolff [5], Toverud and Ender [6], Ellison and Moore [7] and Woo and Chu [8] found that the reserves of human infants, as in the newborn rat, tend to be much lower than in adults, with averages of

10–44 i u per g in different communications (Table 29) In Holland Wolff[5] could detect no vitamin A at all in the livers of nine full term infants out of a group of twenty four The difference between mother and child however was usually much less wide than in the rat A few infants had reserves which equalled or exceeded the average adult level

In more recent investigations however the vitamin A reserves found in both foetuses and newborn infants have been much higher Lewis Bodansky and Shapiro[9] found an average of 134 i u per g for 24 full term infants who had died at birth or shortly afterwards They suggested that this superiority over the reserves found by Wolff was due to a larger intake of vitamin A in the American diet In Finland an even higher average of 285 i u per g was found for 26 foetuses by Skurnik *et al* [10] It is possible that this result was unduly high owing to doubts about the true potency of a vitamin A concentrate used as a standard But the average for the foetuses was certainly higher than the average for a group of adults who had died by accident High reserves in the newborn were also found by Neuweiler[11] Henley Dann and Golden[12] and Marrack[13]

TABLE 29

VITAMIN A IN LIVERS OF HUMAN INFANTS AND FOETUSES

Country	*Date of papers*	*Ref*	*No of cases* Premature	*No of cases* Full term	*Mean vitamin A reserves i u /g* Premature infants	*Mean vitamin A reserves i u /g* Full term infants	*Mean vitamin A reserves i u /g* Mothers
Holland	1932	(5)	18	24	41	44	147
Norway	1935	(6)	47	50	65	39	—
Britain	1937	(7)		11		27	290
China	1939	(8)	23	54	27	10	79
U S A	1943	(9)		24		134	—
Finland	1945	(10)	26	8	285	154	171

It is difficult to understand why the earlier results of Wolff and his followers which agreed with findings in other species should have been superceded in recent work by values so much higher Possibly the reason on the lines that Lewis and his colleagues have suggested can be found in the modern practice of including vitamin concentrates in the diet during pregnancy In agreement with this view the writer and his colleagues noticed a rise in the average vitamin A reserves in Britain between periods centred in the years 1936 and 1942 (Chapter 29) But it is by no means certain whether this attractive theory is correct In any case it must imply that the dietary intake of vitamin A affects the placental passage of vitamin A much more in man than in certain other species including the rat

Before passing from this topic a few other points deserve mention Skur

References p 260

nik [10] suggested that the concentration of vitamin A in the foetal liver reaches a maximum during the earlier stages of pregnancy, and falls as full-term approaches. The findings of Toverud and Ender [8] and of Woo and Chu [9] and Wendt [14] *supported this view. Lewis et al.* [9] observed that vitamin A in the blood of newborn infants fell from an average level of 76 i.u. per 100 ml at birth to only 37 i.u. two and three days after birth, with a recovery to a constant level of 61 i.u. following. Sobel, Besman and Kramer [15] reported that newborn infants were very inefficient, as compared with older children in absorbing vitamin A from oily solution. In spite of these defective absorptive powers, however, Ellison and Moore [7] had found that at an age of 4 *months infants had acquired reserves of vitamin A which were equal,* at least at the period of the observations, to those of children up to 14 years old.

Bovines. Calves have seldom been killed for the estimation of their vitamin A reserves immediately after birth. If they are not allowed access to colostrum, however, they usually die after a few days. In such animals the reserves found at autopsy are usually very low, if measurable. Thus in the In[illegible] and which di[illegible] I[illegible] per g. In three calves which died within 7 days of birth Barron [16] *found no* vitamin A in two of them, and only a trace in the third. Guilbert and Hart [17] found that young calves had reserves much below the adult level. Data obtained by Braun and Carle [18] upon foetuses aborted after 210-277 days by

TABLE 30

VITAMIN A IN THE LIVERS OF FOETAL CALVES AS INFLUENCED BY THE DIET OF THE COW, AND BY DOSES OF 400,000 I.U. OF VITAMIN A TWICE WEEKLY (BRAUN AND CARLE, 1943)

	No of calves	*Vitamin A reserves of liver i u /g* — *Individual cows, where available*	*Average for calves*
Stall fed	7	94, 97, 121	1.7
Stall fed + vitamin A	5	336	14 1
Pasture fed	3	196, 245	6 6
Pasture + vitamin A	5	323, 449	10 7

cows infected by *Brucella abortus* are instructive. Some of the cows received a stall diet low in vitamin A, some received pasture, and with each diet some of the animals were dosed with 400,000 i.u. of vitamin A, as shark-liver oil, twice weekly for the last 2 months of pregnancy. From Table 30 it will be seen that

the amounts of vitamin passed to the foetal liver were always minute when compared with the maternal reserves. It was obvious, however, that the maternal diet affected the amount of vitamin transferred, with average values ranging from 1.7 i.u. per g of liver for calves from undosed stall fed cows up to 14.1 i.u. for calves from stall fed cows which were dosed with shark liver oil.

Even higher doses of vitamin A were subsequently given to pregnant cows by Walker, Thompson, Bartlett and Kon[19] and their results can leave no doubt about the passage of substantial amounts of vitamin A into the foetus when massive doses of the preformed vitamin are given. Some of the calves received the usual routine diet of their farm, with pasture during summer and stall feeding during winter. Others received a diet low in both vitamin A and carotene. Two further groups received the routine diets, but with supplements of 1 000 000 i.u. of preformed vitamin A or 1 500 000 μg of carotene daily. The diets and supplements were all started 3 months before the expected date of calving. For male calves killed immediately after birth the following results were obtained:

	Av. vitamin A in calves' liver i.u./g
Routine diet	2.5
Low in vitamin A and carotene	0.6
Dosed with carotene	4.6
Dosed with vitamin A	142.0

Besides the liver, the blood plasma of newborn calves from normally nourished cows is also low in vitamin A, with levels of 10–20 i.u. per 100 ml as compared with about 100 i.u. in the maternal plasma. Bovine blood plasma normally contains a much higher ratio of carotene to vitamin A than the liver, but only traces are present in the plasma immediately after birth.

Other animals. If the ewes are normally fed, lambs are usually born with low liver reserves of vitamin A. In the author's laboratory Eden[20] found an average of 1060 i.u. per g for eight ewes, and only 11 i.u. per g for their foetuses. Somewhat higher reserves, however, were found by Peirce[21] with an average of 37 i.u. per g for five newborn lambs. Barron[16] examined the livers of 59 lambs, mostly from diseased sheep. In three there was a measurable amount of vitamin, in 11 traces were found, and in 45 no vitamin at all was present.

In newborn kids from goats which had received an ordinary diet Thomas, Loosli and Williams[22] found only 0.2 i.u. of vitamin A per g of liver. In con

liver rose to 33 i u per g

For newborn piglets from normally fed sows Loosli and his colleagues [22] found an average of 16 i u per g of liver Again dosing the mother with pre formed vitamin A greatly increased the amount transferred After daily doses of 400,000 i u the average value for the foetal liver was 119 i u per g Carotene, at the same dosage in international units, did not significantly increase the supply of vitamin A to the foetus

In a newborn pup Van Eekelen and Wolff [23] found 6 i u per g of liver, and in a newborn kitten only 2 i u per g Unusually large amounts of vitamin, however, appear to be passed through the placenta in seals Thus Rodahl and Davies [24] found 120 i u per g of liver in a foetus from a hooded seal (*Cysto phora cristata*) and 240 i u, in a foetus from the Greenland seal (*Phoca groen landica*)

It seems possible that the apparent disparity between species in the pla cental transfer of vitamin A, as evidenced by the low transfer in rats as compared with the higher transfer sometimes found in humans, can be ex

[illegible]

mothers obtain preformed vitamin A from their diet of fish, is in accordance with this view

The Blood Vitamin A During Pregnancy

We must now go on to the discussion of levels of vitamin A and carotenoids in the blood of the mother during pregnancy The first point which has been clearly established is that the level of vitamin A, and still more the level of carotenoids, is lower in the foetal than in the maternal circulation A second point is that during the final stages of pregnancy the vitamin A level in the maternal blood decreases considerably. This change, however, is only temporary, and the normal level in the blood is regained after parturition without special dosing with vitamin A

Vitamin A in the maternal and foetal blood

In conformity with the idea that the passage of vitamin A through the placenta is at least partially restricted several workers have found that vitamin A is much lower in the foetal blood stream than in the maternal The disparity for carotenoids is even greater In 1937 Gaehtgens [26] found an average of 70 μg for carotenoids in human maternal blood and 20 μg for foetal blood He also reported that vitamin A was much higher in maternal than in foetal blood, but the methods of analysis then available only allowed a rough comparison Ten years later an extensive investigation by Lewis *et al* [27] gave the following results

	No of cases	*Vitamin A i u /100 ml plasma*	*Carotenoids μg/100 ml plasma*
Maternal 3rd trimester	62	91	127
Umbilical vein	12	61	16
Umbilical artery	12	58	17

It will be seen that vitamin A was some 30% lower in the foetal circulation than in the maternal, but that carotenoids were reduced in the foetus to only about one eighth of the maternal level. According to Hoch [28] the difference between carotene concentrations in the foetal and maternal blood is even greater than comparisons of the total yellow colour would indicate since the ratio of non carotene pigments to carotenes is higher in the foetus than in the mother

Lewis and his colleagues [27] found that daily doses of 10 000 i u of vitamin A or carotene during the last few months of pregnancy had no effect on their levels in the foetal blood at birth

The fall in the maternal blood vitamin A during the 3rd trimester of human pregnancy

The first description of this interesting phenomenon appears to have been given by Lund and Kimble [29] In pregnant women they found that the level of vitamin A in the blood plasma [illegible] ours [illegible] the [illegible] the stage of pregnancy but both vitamin A and carotenoids were influenced by the amounts available in the diet At about the same time similar results were obtained by Bodansky Lewis and Lillienfeld [30] who agreed that vitamin A fell without a corresponding decrease in carotenoids Their results follow

References p 260

Duration of pregnancy	No of cases	Mean vitamin A i u /100 ml plasma	Mean carotenoids µg/100 ml plasma
Under 6 mths	70	105	112
Over 6 mths	62	91	150

Daily supplements of 10,000 i u of vitamin A, which had been found ineffective by Lewis *et al.* [27] in raising vitamin A levels in the foetus, could nevertheless compensate for the decline in the blood during the 9th month of pregnancy Thus for 26 dosed women the average level was 122 i u, as compared with 93 i u in 27 undosed women Daily supplements of 10,000 i u of carotene similarly raised the plasma vitamin A to 123 i u, and at the same time increased the carotenoids to 243 µg, as compared to 127 µg without dosing

Cayer, Crescenzo and Cody [31] compared the average vitamin A contents of the blood plasma with the total lipoid contents Their results, which show some degree of parallelism, were as follows

Stages of pregnancy	No of cases	Vitamin A i u /100 ml	Lipoids mg/100 ml
1–3 mths	16	106	607
4–6 mths	19	123	760
7–9 mths	26	102	691
Post parturition	16	110	843

Probably the fall in the blood vitamin A in the third trimester is related to other changes in the blood There is some evidence, however, that these changes are not universal, such as would be produced by a mere dilution of all the blood constituents We have already mentioned that blood carotenoids do not appear to decline in parallel with vitamin A Vitamin E, another fat-soluble vitamin, is reported to increase in the blood plasma during pregnancy, with the average level at term 65% greater than before the start of pregnancy [32] According to Gaehtgens [33] vitamin A is excreted in the urine in about 25% of pregnant women, which at least suggests changes in vitamin A metabolism other than dilution, can frequently occur Finally there is evi- [34, 35]
de

without dietary changes, it seems obvious that the decrease in ... during later pregnancy reflects a change in the equilibrium between the liver and the blood plasma There is no evidence that the maternal reserves of vitamin are seriously depleted at this point.

The fall in vitamin A and carotene in the cow before parturition

Evidence of a decrease in the plasma vitamin A, similar to that seen in human pregnancy but accompanied by a parallel decline in carotene, has also been reported for cows [19, 36, 39]. Figure 13 is based on data obtained by Sutton, Kaeser and Solner [40], during observations on 28 cows Sharp falls in both vitamin A and carotenoids are seen during the three weeks before parturition followed by recoveries during the first two weeks *post-partum* The high ratio of carotene to vitamin A, typical of bovines, will be noticed and the corresponding difficulties in analysis must not be overlooked (Chapter 7) Again we must consider how far the changes are specific, and how far they are merely a minor reflection of other, more important, metabolic changes Goodwin and Wilson [41] who have recently confirmed the early findings, favour the second view Thus they point out that plasma lipoids, calcium and phosphorus are also lowered at the time of parturition in cows In contrast to the finding for the human moreover, there is some evidence that the level of vitamin E in the plasma is reduced [42]

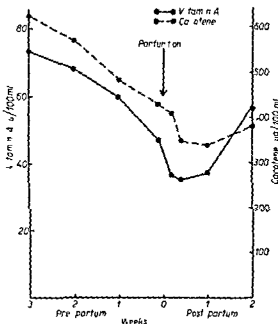


Fig 13 Decreases in vitamin A and carotene in the blood plasma of cows during the last weeks of pregnancy and their return towards normal after calving (after Sutton *et al*)

The Transfer of Vitamin A via the Colostrum

As the end of pregnancy approaches colostrum accumulates in the mammary glands This turgid, viscous material is rich in globulins, and during the early stages of its formation may perhaps be regarded as a concentrated form

of blood plasma After parturition the colostrum is blended with the first flow of milk, which washes out the colostrum during the first two or three days of lactation The colostrum is much richer in vitamin A than the subsequent milk, and also much more variable in its vitamin contents between different individuals It is also richer than milk in other vitamins, and is important to some animals for the immunological properties of its globulins Thus if calves are denied colostrum they frequently die, within a few days of birth from a form of diarrhoea known as 'white scour" In view of the importance of colostrum for the bovine we may deal with this species first

Bovine colostrum In 1933 Dann[43] studied the secretion of vitamin A and carotenoids in cows' colostrum The highest concentrations were always found on the first one or two days and had usually decreased to less than one tenth of the original level within a week Although Dann's values were given in Lovibond units we may calculate that the vitamin A contents of the early colostrum from his 14 cows varied from 180 to 1600 i u per 100 ml, and the carotenoid contents from 20–680 μg At the time Dann claimed that his richest colostra contained 70 times more vitamin A than 'standard milk", but the value considered typical for milk was certainly much too low If we take milk as containing 140 i u of vitamin A per 100 g *it seems that a ratio of 11 or 12 would have been more appropriate*

Dann calculated that a calf might receive 10 lbs of colostrum if allowed free access to suckle With a colostrum of average potency this would provide 27,000 i u of vitamin A and 22,500 μg of carotenoids In contrast the calf s liver at birth would weigh 800 g and might contain a total of only 400 i u of vitamin A Although the globulins of colostrum are now recognised as the components most vital for the calf's survival it is clear that, as a further benefit, a contribution of vitamin A greatly in excess of the animal's existing reserves is also received

Subsequent estimations of vitamin A and carotenoids in bovine colostrum [44-47] have agreed fairly well with Dann's findings both on the wide range of spread of potencies and on average values (Table 31) Since the vitamin A and carotene contents of the lacteal secretion at first fall rapidly as milk replaces colostrum and then decline more slowly as lactation proceeds it is difficult to calculate an exact ratio between the concentrations in the [illegible] both forms of colostrum and in [illegible] secretion is influen[illegible] are not understoo[illegible] instructive to calculate averages from data obtained by Sutton *et al* [44] on colostrum and milk from five breeds of cows (Table 32) For first day colostrum the average for vitamin A was 799 i u per 100 ml and for carotene 473 μg For 20th day milk the corresponding averages were 143 i u

and 41 μg Colostrum therefore appears to be 5 times richer than milk in vitamin A, and 12 times richer in carotenoids The superiority of colostrum is not due to a higher content of fat, and the ratios just given are somewhat increased if the vitamin A and carotenoid concentrations are calculated on the weight of fat secreted

There is plain evidence that the restriction of cows to a diet low in caro-

TABLE 31

AVERAGE VITAMIN A AND CAROTENOID CONTENTS OF EARLY COLOSTRUM AND LATE MILK IN VARIOUS SPECIES THE DIETS OF THE RUMINANTS WERE PRESUMABLY ADEQUATE IN CAROTENE, BUT WERE NOT SPECIALLY FORTIFIED WITH VITAMIN A

Species	Colostrum Vitamin A i u /100 ml	Colostrum Carotenoids μg/100 ml	Milk Vitamin A i u /100 ml	Milk Carotenoids μg/100 ml	Reference
Human	380	160			Dann (1936)[57]
„	650	240	200	26	Macy (1945)[59]
„			133	15	Kon (1950)[58]
Cow	730	210			Dann (1933)[43]
„	630	420			Luecke (1947)[45]
„	680	440			Blakemore (1947)[46]
„	800	470	143	41	Sutton (1947)[44]
„	870	184	132	30	Chanda (1953)[47]
„			156	31	Booth (1933)[43]
Sheep	1290	—			Underwood (1944)[50]
„	2300	—			Peirce (1947)[51]
„	1830	—			Eden (1948)[52]
„			136	—	Weir (1949)[53]
Goat	1210	—			Thomas (1947)[22]
„	1520	—	134	—	Chanda (1953)[47]
Sow	250	—			Braude (1946)[55]
„	560	—			Thomas (1947)[22]
„	480	—	176	—	Bowland (1949)[54]

TABLE 32

VITAMIN A AND CAROTENOIDS IN THE COLOSTRUM AND MILK OF COWS OF DIFFERENT BREEDS (SUTTON *et al* 1947)

	No of cows	Vitamin A i u /100 ml 1st Day colostrum	Vitamin A i u /100 ml 20th Day milk	Carotene μg/100 ml 1st Day colostrum	Carotene μg/100 ml 20th Day milk
Ayrshire	6	606	159	373	25
Guernsey	8	929	70	864	52
Holstein	9	562	150	289	30
Jersey	7	479	173	335	43
Brown Swiss	5	1158	170	497	41
All breeds	35	799	143	473	41

References p 260

tene and vitamin A may reduce both substances in the colostrum Massive doses of vitamin A may increase its concentration in the colostrum above the normal level Massive doses of carotene may increase the carotenoid contents of the colostrum, but will not raise vitamin A above the normal level Thus Spielman *et al* [45] found that daily doses of 1,000,000 i u of preformed vitamin A increased the average vitamin A concentration in the colostrum from 1200 i u to 2300 i u per 100 ml The same dose of carotene had little influence on either the colostral vitamin A or carotenoids In experiments by Walker *et al* [19] the influence of vitamin A in increasing vitamin A in the colostrum was confirmed, and the effect of a diet low in carotenoids was clearly demonstrated With such a diet the colostral fat contained only 133 i u of vitamin A and 13 μg of carotenoids per g, as compared with 203 i u and 64 μg under normal conditions of management

The effect of colostrum in increasing the levels of vitamin A and carotenoids in the blood plasma of newborn calves has been amply established by Moore and Berry [49] The average plasma vitamin A for over 40 calves, of 4 different breeds, was 11 2 i u per 100 ml before suckling and 50 i u on the first day following suckling For carotenoids the corresponding values were 2 4 μg and 25 μg Only minor increases occurred during the following six days, during which the animals received milk The levels of vitamin A and carotene in the calf s plasma increased very little, and very slowly, if no colostrum was allowed, and only milk was given from birth

Colostrum of other farm animals

Sheep colostrum has been studied by Underwood and Curnow [50] Peirce [51], Eden [52] and Weir *et al* [53] The data of all these workers indicate that the colostrum of this white fatted animal tends to be slightly richer than that of the cow in vitamin A, but that only traces of carotenoids are present (Table 31) Eden has shown in the author's laboratory, that when lambs receive colostrum there is a temporary increase in the vitamin A level in their blood This is followed by a return to the level found at birth in parallel with the decrease of vitamin A as colostrum changes to milk (Fig. 14).

In goats colostrum Thomas, Loosli and William [22] found about as much vitamin A as in sheep colostrum, the concentration could be trebled by giving massive doses of the vitamin during pregnancy Chanda's data [47] for colostrum from undosed goats were in close agreement

In sows' colostrum Thomas and his colleagues found only half the vitamin A found in the sheep and goat, but again the concentration could be trebled by massive dosing with the preformed vitamin For the colostrum of undosed sows Bowland *et al* [54] obtained a very similar average, but an even lower value found by Braude, Kon and Thompson [55] emphasised the inferiority of sows' colostrum to those of the cow and sheep

Buffalo colostrum, according to Narayanan *et al* [56] contains about the same concentration of vitamin A as cow colostrum

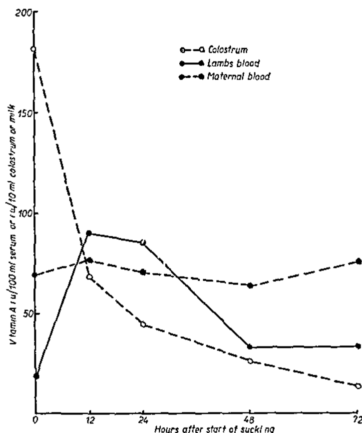


Fig 14 Average vitamin A levels in the blood plasma of newborn lambs and of their mothers and in the colostrum at different times after the commencement of suckling (Eden)

Human colostrum In the human relatively little colostrum is secreted, and failure to consume it does not involve such disastrous consequences as may ensue in other species particularly the bovine As in farm animals, however, human colostrum contains vitamin A in higher concentrations than are later available in the milk Dann [57] found that vitamin A averaged 377 i u and carotenoids 158 μg in the colostra of 111 women, with a somewhat higher average for vitamin A in coloured than in white women. The value quoted by Dann for milk related to specimens collected during the first 14 days after parturition, which must still have contained twice the level of vitami

colostra were

times richer ii

colostrum as such in their informative report on human milk, but colostral

References p 260

fat with 119–159 i u. of vitamin A per g was much richer than typical milk fat, at the 17th week of lactation, with only about 30 i u. For carotenoids the colostral level was 26 1–33 1 μg as compared with about 4 3 μg in the milk fat.

According to Macy and her colleagues [59] vitamin A was highest in human colostrum on the third day, with 653 i u per 100 ml, and carotenoids were highest on the first day with 241 μg per 100 ml The values are obviously of the same order as those of Kon and Mawson if we assume that the colostrum examined by the British workers contained about 5% of fat. The decline of vitamin A and carotenoids as lactation advances is shown in Fig 15

It appears that human colostrum has about half the concentration of bovine colostrum both in vitamin A and in carotenoids.

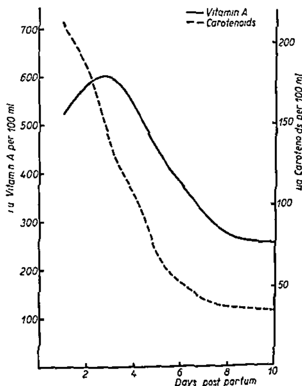


Fig 15 Average vitamin A and carotenoid levels in human colostrum milk collected from numerous women at different times after parturition (after Macy *et al*)

THE TRANSFER OF VITAMIN A VIA THE MILK

As full lactation is established the higher concentrations of vitamin A and carotenoids, typical of colostrum, are rapidly reduced At the same time the volume of secretion greatly increases, with the result that the daily outputs of vitamin and pigments are substantially raised.

Cows' milk A prolific literature exists on the vitamin A contents of cows' milk and butter fat. In addition to the stage of lactation the nature of the diet has an important influence, and in turn the diet is usually

influenced by the season of the year Thus during winter cows may be fed upon cattle cakes and poorly preserved hay low in carotenoids but in summer they have access to lush pastures Breed also affects the carotenoid contents of the milk Thus under similar dietary conditions yellower milk is given by the Channel Island breeds than by most other breeds It is very difficult therefore to deduce single values for vitamin A and carotenoids which can be taken as typical of average milk or butter We may consider however, that the natural diet of the cow is grass which provides carotene greatly in excess of the vitamin A that is formed (Chapter 13) Data on pasture fed cows of five different breeds was obtained in the careful work by Sutton *et al* whose results on colostrum have already been mentioned (Table 32) It will be seen that in milk of the 20th day vitamin A ranged from 70 to 173 i u per 100 ml according to breed and carotenoids from 25 to 52 μg Averages for 35 cows of different breeds were 142 i u and 38 μg

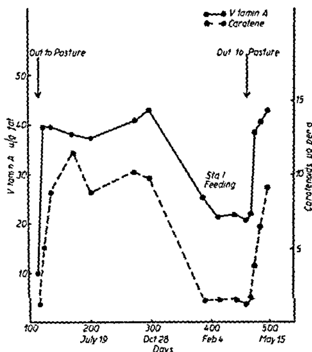


Fig 16 Variations of the vitamin A and carotenoid contents of butter fat according to the season of the year and the access of the cows to pasture (after Booth *et al*)

If we regard the cow as normally fed when at pasture it is clear that the removal of carotenoids from the diet can greatly reduce the vitamin A and carotenoid contents of the milk This important point seems first to have been established in 1921 by Hughes Fitch and Cave [60] and was soon confirmed by others [61, 62] One of the first of many systematic studies of the effect of stall feeding as opposed to pasture was made in 1933 by Booth Kon,

References p 260

Dann and Moore[63] The pronounced variations between winter and summer values, both for vitamin A and carotenoids, are shown in Fig 16 For nine summer collections average values of 156 i u of vitamin A and 31 μg of carotenoids per 100 ml of milk may be calculated by modern methods as compared with 79 i u and 5 3 μg for six specimens of winter milk It will be noticed that during stall feeding vitamin A was only reduced to about half the summer level, but that carotenoids fell to about one sixth of the summer level Similar differences were observed by Baumann and Steenbock[64], but their values for vitamin A were based on the uncorrected absorption at 328 mμ, and hence were unduly high

The failure of comparatively small supplements of carotene to prevent the fall in the milk carotenoids was discussed by Watson *et al*[65] who reported that the inclusion of 25% of dried grass in a winter diet failed to cause any improvement This finding may presumably be explained by the very low efficiency with which carotene is utilised by the bovine, as already mentioned According to Atkeson *et al*[66] the average daily carotene consumption of three cows was 3 5 g, and of this only 0 15% was recovered in the milk as vitamin A and 0 086% as carotene Watson and his colleagues mentioned that it seemed impossible to raise the carotenoid and vitamin A contents of milk above a fixed "ceiling", no matter how much carotene was given This ceiling varied with breed and was higher for Shorthorn cows than for Ayrshire cows Probably these findings are also related to the very low utilisation of carotene

Preformed vitamin A is seldom given to dairy cows under practical conditions but when it is administered in large doses for experimental purposes it appears to pass into the milk more readily than carotene Thus Kalyankrishnan *et al*[67] found that large daily doses of shark-liver oil greatly increased vitamin A in the milk fat of cows whose only other source of the vitamin was 10 lbs of grass Their findings were as follows

Dose of vitamin A i u	*Vitamin A in milk fat* i u /g
0	13–14
100 000	28–51
200 000	57–70

The effect of a short period of massive dosing with various different preparations of vitamin A on its level in the milk of cows was studied by Sobel [illegible][69] When the vitamin was given orally either in oily

we ... the peak occurred after only
10 ... g
... d in the milk corresponds to
only a minute fraction of the carotene supplied by pasture it is equivalent to a much more significant fraction of the body's total reserves of vitamin A. If we were to restrict a high yielding milking cow to a diet virtually free from vitamin A and carotene we might expect the animal to continue to secrete about 1 kg of milk fat daily, containing about 20 i u of vitamin A per g The total daily secretion would thus be 20 000 i u In comparison the liver might weigh 5 kg, and contain 150 i u of vitamin A per g making a total of 750,000 i u [69] The liver reserve thus appears only capable of maintaining vitamin A in the milk, at its winter level, for a period of about six weeks The drain on the carotene contents of the body is presumably even more severe, and it can only be expected that the milk fat will become paler in colour soon after feeding on a diet low in carotene has been started

Carotenoids in cows' milk This point seems appropriate for discussion of the identity of the carotenoids present in cows' milk and colostrum As early as 1914 Palmer and Eckles [70] applied chromatographic absorption to the examination of the carotenoids of milk fat According to Gillam and Heilbron [71] α and β carotenes predominate, with smaller amounts of cryptoxanthin and lycopene Willstaedt and With [72] reported that the pigments were almost exclusively carotene More recent studies by Thompson *et al* [73] however have shown that the proportion of carotenes to other pigments may vary from 25 to 90%, with higher percentages for milk fats rich in carotenoids than for the less yellow specimens But low percentages of carotenes were found in only a few instances, and for 200 specimens of milk the mean percentage of carotenes presumably mainly β, was 74%

Thompson and his colleagues also made the interesting observation that the concentration of carotenoids in milk fat varies widely according to whether the fat is separated from the whole milk or from separated milk or whey The variation went parallel with cholesterol contents of the fat, but not with vitamin A, and was related to the size of the fat droplets Presumably carotenoids and cholesterol, but not vitamin A, tend to be concentrated on the membrane of the fat globule The striking results obtained on five different milk fractions are given in Table 33 It will be noticed that the concentration of carotenoids, per unit of fat, was more than 10 times greater in whey than in milk Possibly these findings may give a clue to understanding why the ratio of carotene to vitamin A is often much higher in bovine blood plasma than in either the colostrum or the milk

The same workers also reported that virtually all the vitamin A present

References p 260

in cows' milk and colostrum is in the esterified form According to Chanda [74], however, bovine colostrum may have up to 10% of its vitamin A as the free alcohol and milk up to 5% as the alcohol

TABLE 33

THE VARIATION OF THE CAROTENE CONCENTRATION PER UNIT OF FAT IN VARIOUS MILK FRACTIONS (THOMPSON *et al*, 1949)

Fraction	*Radius of fat globule μ*	*Cholesterol mg/g fat*	*Carotenoids μg/g fat*	*Vitamin A i u /g fat*
Cream	1 68	2 8	9 5	25 3
Milk	1 41	3 3	9 8	24 7
Buttermilk	0 89	6 0	12 2	24 2
Separated milk	0 51	36 0	65 4	21 6
Separated whey	Too small to measure	48 0	111 0	26 2

Milk of other animals Data on the vitamin contents of the milk of the sheep [32], goat and sow are compared with data for human and cows' milk in Table 31 The values for the human subject, cow and goat refer to about the 20th week of lactation, those for sheep to the 8th week, while the stage of lactation in the sow is unknown Although comparisons are difficult, in view of the consistent fall in vitamin A in early lactation, it seems that under comparable conditions there is remarkably little difference between the vitamin A contents of the milk of all these species This generalisation appears to apply equally to the animals with yellow and with white body fat It would not seem far wrong to say that any of the milks, collected during the middle of lactation from a group of normally nourished animals may be expected to average about 140 i u of vitamin A per 100 ml

The fat contents of the milk of all the species which have just been mentioned is usually not far removed from 4% There is at least a suspicion that the milk of certain other animals may contain both more fat and more vitamin Thus Houston, Kon and Henry [75] found that rat milk contained 14% of fat and 330 i u of vitamin A per 100 ml Carotenoids were virtually absent Guinea pig milk contained 8% of fat, with 230 i u of vitamin A and 11 μg of carotenoids per 100 ml It is possible, of course, that in these small animals, with only short periods of lactation the fall in vitamin A from the high values of the colostrum and of the early milk, may be less pronounced than in larger animals

Interesting findings by Indian workers on the milk of the buffalo which is locally important as a dairy animal, must also be mentioned The milk of this animal, unlike that of the cow, is almost colourless The fat content is usually

8% and various estimates of the vitamin A contents range from 120 to 330 i u per 100 ml [76, 77, 78]

Human milk The most complete study of the vitamin A contents of human milk is probably that of Kon and Mawson [58] During the collection of serial specimens from numerous women all the complications of the problem were recognised and action was taken to avoid misleading results Thus the need of uniformity in sampling was realised At the same collection the results might vary widely between the two breasts and between milk obtained by partial or complete removal from the same breast Moreover the vitamin A contents of the milk as already mentioned decreased continuously during the first 20 weeks of lactation By completely emptying at least one breast 4 hours after the last suckling and by comparing the results of different groups at a standard time after parturition however it was possible to obtain much valuable information For women in the 20th week of lactation vitamin A averaged 133 i u per 100 ml and carotenoids [illegible] ppeared to [illegible]

[illegible] Macy and her colleagues [59] For nearly two hundred specimens of milk from 69 mothers who had been lactating for 15 to 362 days an average of about 200 i u per 100 ml was found for vitamin A and 25 5 µg for carotenoids This higher value for carotenoids in comparison with Kon s average may well be linked up with the common observation that in America the level of carotenoids in the blood plasma tends to be much higher than in England (Chap 29) It should be noted however that whereas the average concentrations of vitamin A in human milk and in blood plasma are of the same order (say 140 i u and 130 i u respectively) the concentration of carotenoids in milk is much lower than in blood plasma (say 100 µg as against 20 µg) This difference seems to reflect the greater ability of vitamin A as compared with unchanged carotenoids in passing barriers in this instance the cells of the mammary gland

Evidence of the effect of nationality and presumably of the corresponding dietary habits on the vitamin A contents of the milk was also obtained in early work in Batavia by Meulemans and de Haas [79] For milk obtained during the second quarter after parturition the following averages were found

Nationality	*No of samples*	*Vitamin A i u /100 ml*	*Carotenoids µg/100 ml*
Batavian	125	34	13
Chinese	116	43	16
European	22	104	28

References p 260

These results suggest that human milk no less than cows milk may contain abnormally low amounts of both vitamin A and carotenoids when the diet is deficient in these factors

In regard to the effect of dosing in increasing the vitamin A in the milk above its normal level we again see much the same picture as in other species Kon and Mawson[58] found that small supplements of vitamin A during pregnancy usually 4000 i u daily had no effect on the vitamin A contents of the milk On the other hand a massive dose of 240 000 i u just before parturition or daily doses of 24 000 i u daily during the first 9 days of lactation considerably increased the level of vitamin A in the milk Thus on the 9th day of lactation the average concentration of vitamin A in the milk fat of mothers who had received large daily doses was 120 i u per g as compared with 70 i u in the milk fat of undosed mothers Sobel Rosenberg and Kramer[60] confirmed the effect of massive dosing in raising vitamin A in the milk fat and found greater increases when the vitamin was given as an aqueous emulsion than when it was given in oily solution The effect of a single dose on the vitamin A level in the milk was temporary, as in the blood plasma but the peak occurred 12 hours after dosing as compared with after 3 hours in the blood

The output of vitamin A during human lactation represents a substantial proportion of the dietary intake Kon and Mawson[58] calculated that in their investigation women who received 2500 i u of vitamin A daily in their diet together with small amounts of carotene secreted about 1000 i u of vitamin A in their milk The transfer to the milk thus amounted to nearly 40% of the intake The average liver reserves in well nourished populations however should be able to guarantee the supply of vitamin A to the milk for much longer periods than the six weeks which we have calculated for the cow Thus the typical British reserve of about 500 000 i u should suffice for over a year It would be foolhardy all the same not to make sure that both expectant and nursing mothers receive adequate supplies of vitamin A

Human milk carotenoids The yellow pigments of human milk differ from those of bovine milk in containing a much smaller proportion of carotenes In 1914 Palmer and Eckles[81] found about equal proportions of carotene and xanthophylls in two specimens of human fat but other workers found only small proportions of xanthophylls[67, 82] According to With and Friderichsen[83] however β carotene contributed only one third to one quarter of the total carotenoids of human milk Truka Tuzson[84] found an even smaller proportion

Extensive estimations by Thompson Kon and Mawson[85] were made by a modern chromatographic method on specimens of milk fat from 75 women On an average they found that α and β carotenes made up 23% of the total

pigments lycopene 9% an unknown pigment 21% and lutein (xanthophyll) 47% The predominance of pigments other than carotenes has thus been clearly proved We shall see in Chapter 29 that pigments other than carotenes also make a substantial contribution to the yellow colour of human blood plasma The ratio of carotenes to non carotenes in blood however is probably greater than in milk

THE TRANSFER OF VITAMIN A FROM BIRD TO CHICK

As might be expected our most extensive information of the transfer of vitamin A to the egg relates to the domestic hen The growth promoting activity of its yolk was established in the early days of vitamin research [86] and later the presence of preformed vitamin A was established by von Euler and Klussmann [87] According to Gillam and Heilbron [88] the activity of the yolk can be ascribed to the combined actions of the preformed vitamin carotene and cryptoxanthin The provitamin constituents however make only small contributions either to the total vitamin A activity or to the total yellow colour The predominant pigment under normal conditions of nutrition is the inactive xanthophyll In Chapter 1 we have already referred to the early experiments by Palmer and Kempster on the successful hatching of eggs with colourless yolks which had been obtained by giving hens a diet free from carotenoids but containing cod liver oil

The influence of dietary vitamin A on the vitamin A content of the egg was studied by Bethke *et al* [89] They found by biological tests that the addition of 2% of cod liver oil to the diet of hens otherwise low in the vitamin increases the content of the eggs about five fold Cruickshank and Moore [90] found by the antimony trichloride method that the eggs from normally fed hens given a diet containing yellow maize and 1% of cod liver oil averaged about 300 i u of vitamin A per egg or 55 i u per g of fat When a diet low in carotenoids and without cod liver oil was given each egg contained only about 150 i u The inclusion of 10% of cod liver oil in such a diet gave eggs containing 340 i u and doses of vitamin A at the rate of 250 000 i u daily brought the contents up to nearly 1000 i u It was emphasised however that only 2% of the dietary intake of vitamin A was passed into the egg when 10% of cod liver oil was given and 0 2% when the concentrate was administered

Vermes Meunier and Raoul [91] found that the eggs of well nourished hens contained 300–800 i u of vitamin A each or about 50 i u per g of yolk About one third of the vitamin A originally present in the egg was found in the liver of newly hatched chicks but this reserve completely disappeared after only 8 days of subsistence on a diet deficient in the vitamin Lissot and

References p 260

Candroit[92] concluded that eggs must contain at least 350 i u for hatching to be successful

Further extensive studies of the transfer of vitamin A from the yolk to the embryo were made by Parrish *et al*[93] The effect of differences in the vitamin A contents of the hen's diet on the vitamin A contents of the embryo became increasingly evident as development progressed Thus 12 day-old embryos from the eggs of hens which received diets containing 1800 i u, 3300 i u or 12,300 i u per lb each contained 1 20 i u, 1 96 i u or 2 42 i u, with only a twofold difference between the first and third groups At 21 days, however, the newly hatched chicks, with the residual yolks removed contained 10 1, 60 7 and 101 i u respectively, with a ten fold difference between the outer groups In eggs from the middle group the estimations were extended to the yolk and extra embryonic tissues Each fresh egg contained about 220 i u of vitamin A, and each newly hatched chick had 61 i u in its body and 97 i u in its yolk sack. Of the body vitamin A 48% was located in the liver The loss of vitamin during hatching was calculated at 28%, but a similar loss was also observed in eggs which had not been fertilised

Parrish and his colleagues[94, 95] have also reported the surprising finding that about 80% of the vitamin A content of the fresh egg is present as the free alcohol During the development of the embryo, however, the percentage of ester increases throughout the egg Thus the egg yolk contained 23% of its vitamin A as esters before incubation started, but 72% on the 18th day The liver of the newly hatched chick had 87% of its vitamin esterified No corresponding esterification of vitamin A was observed when unfertilised eggs were incubated

General Conclusions

Before we try to interpret the significance of the findings which have been reviewed in this chapter we must bear in mind one final point which is obviously of the highest importance Studies on various animals which will be [illegible] vitamin A [illegible] reproduc- [illegible] breed or [illegible] malfor- [illegible] of the young offspring may soon [illegible] interest ing, therefore, to look for evidence of mechanisms which may assist in the transfer of vitamin from mother to young

Such mechanisms certainly exist Thus we have seen that the colostrum provides a source of the vitamin which is available immediately after parturition The milk continues the supply in less concentrated form, but in

total amounts which are adequate for the young animal From the evidence of dairy cows during winter feeding we know that the secretion of vitamin A in the milk is continued in spite of the absence of adequate amounts of vitamins or provitamins in the diet Presumably the vitamin is mobilised from its stores in the liver so as to maintain a minimal level in the milk

In the opposite direction dosing with preformed vitamin A provided it is at a sufficiently high level will raise the vitamin contents of the milk and colostrum above their normal levels The extent of the increases, however will be very small compared with the dose of vitamin which is supplied The vitamin will be diluted down by the process of lactation and not concentrated up It seems a matter of personal choice whether we are to regard this limitation as a means of protecting the suckling against an unwanted excess of the vitamin or merely as an indication that the mammary glands are to some degree resistant to the passage of the vitamin

During gestation moreover a similar limitation on the transfer of the vitamin is imposed by the placenta which seems to act as a filter holding back the vitamin rather than as a mechanism for aiding its passage Thus it is clear that the level of vitamin is always lower in the foetal blood than in the maternal blood Perhaps the most striking instance of this restriction is seen in the pregnant rat when the administration of enormous doses of preformed vitamin A to the mother will not cause more than traces to appear in her foetuses Similarly in hens which are heavily dosed with vitamin A the amounts which are transferred to the egg yolk are relatively small The livers of newborn calves are very low in the vitamin except when their mothers have been given massive doses Only in human babies and in young seals can the presence of moderately large reserves be considered as commonplace As already suggested the exceptionally high transfer of vitamin to the foetal seal may be due to the inclusion of large amounts of the preformed vitamin in the diet In the human low foetal reserves have been reported rather more often than high reserves

cause a fall of vitamin A in the blood This fall may well be considered as a parallel to the decline in vitamin A in cows milk during winter feeding On the other hand an increase of the intake of vitamin A will be absorbed by the liver and will only show in the blood if the absorptive power of the liver is temp[illegible]

diet

these

References p 260

of vitamin would demand. Large doses of carotene can increase the carotenoid content of the milk, as they increase the level in the blood But the related increase in vitamin A may not be large enough, or rapid enough, to exceed the absorptive powers of the liver.

The foetus and lactating offspring therefore appear to come under the control of the *general system which gives the liver a central position in the distribution* of vitamin A, and which normally limits the supply of vitamin to other parts of the body.

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CHAPTER 22

Vitamin A and Vision

The role of vitamin A in vision, and particularly in scotopic vision at low illuminations, is in the author's opinion one of the most fascinating subjects in the whole range of biochemistry It may be an inspiring thought, to workers in the field, that Man's knowledge of the existence of the stars, and of the vast universe which appears in the heavens each night, comes in the first place from the stimulation by their light rays of delicately poised molecules of vitamin A

In Chapter I we have seen that the affliction of night blindness, and its cure by liver or liver oils, was known to the ancients, in centuries long before the existence of vitamins had been discovered The steps which have led from these early beginnings up to our present satisfactory knowledge of the role of vitamin A aldehyde in vision, as the prosthetic group of rhodopsin, have demanded powers of investigation of a very high order As a first difficulty most of the substances under investigation are sensitive to light. Many observations, therefore, must be made in subdued illumination. Secondly these substances are usually present in the eye only in small traces, which do not allow their isolation and identification in the pure form Their recognition must therefore depend almost entirely on spectroscopic evidence. Fortunately it has been possible to demonstrate that authentic vitamin A, from external sources, can replace the native vitamin A of the retina in many of its reactions, as demonstrated *in vitro*

In spite of the difficulties of the field, however, the brilliant researches of Wald, Morton and others have given us a very full and detailed picture of the physiological and biochemical significance of vitamin A in the retina It is indeed ironical that we should know so much about the biochemistry of the traces of vitamin A which are situated in the eye, and relatively so little of the biochemistry of the much larger amounts which are present in the rest of the body.

Perhaps the anomaly may be partly explained by the interest evoked by visual purple, now known as rhodopsin, long before even the existence of

References p 276

vitamin A was known Thus research in this field started nearly a century ago and has been assisted in more recent years by accurate spectrophotometry Zoologists and comparative biochemists have been attracted quite apart from the vitamin story by the interesting minor variations which can be noticed between the visual pigments found in different species With the nature of the protein part of the molecule varying for each species many slightly different photosensitive pigments have been detected and have been characterised by minor differences in their absorption spectra Wald sees a parallel in the haemoglobins which have the same prosthetic haem combined with globins which vary with species As a further field of interest vitamin A_2 forms a second set of pigments known as porphyropsins A mass of knowledge has thus accumulated which provides a wealth of information for those interested in differences between species

In this book pressure of space prevents a comprehensive review of all the modified forms of visual purple found in different species Neither can a complete account be given of all the intermediate products formed during the bleaching of visual purple or of artificial compounds made for the purpose of simulating its properties For information on these points the reader is referred to articles by Wald[1] Lythgoe *et al*[2,3] Collins and Morton[4] and Collins[5]

Early Developments

It is difficult after the lapse of years to trace the exact steps by which at first the general importance of vitamin A for vision was recognised and later the specific role of vitamin A aldehyde as a component of rhodopsin was proved But it is clear that progress was made down converging paths rather than along a single track

Vitamin A deficiency and hemeralopia One of the earliest associations to be established was that between defective adaptation to vision in the dark (hemeralopia or nyctalopia) and the other effects of vitamin A deficiency Thus in 1863 Bitot[6] recognised the relationship between hemeralopia and the spots on the conjunctiva which bear his name In 1876 Snell[7] observed similar spots in children of Sheffield who were brought to him by their parents because they could not see in the streets after dark Moreover the therapeutic value of cod liver oil in curing both the hemeralopia and the spots was recognised even at this early period Snell considered that the diet of his patients was adequate but nevertheless his treatment consisted of the administration of cod liver oil and steel Possibly the latter was given in deference to Sheffield s main industry

The recognition of both hemeralopia and lesions of the conjunctiva as effects of vitamin A deficiency however had of course to await the discovery of the vitamin as described in Chapter 1

Chemical studies on visual purple

Wald [8] gives credit for the discovery of visual purple to Franz Boll in 1877 [9]. This pioneer noticed that visual purple obtained from a frog's retina turned yellow on treatment with acid He inferred from this change that the purple was derived from the yellow pigment which abounded in the pigment epithelium This led his colleague Capranica [10] to conclude that the pigment was lutein a term which at that time covered both carotene and xanthophyll In view of the scanty knowledge of the carotenoids which was available in 1877 these early findings seem to have been surprisingly well in line with more modern developments Thus the original conception of visual purple as a carotenoid derivative in the broadest sense has stood the test of time Modern workers, however, agree that the actual colour of the complex is red rather than purple

In 1878 Ewald and Kuhne [11] pointed out the error of Boll's hypothesis, as applied in its simplest form By various tests it was shown that the pigment obtained by acidification of the retinal extract was quite different from that of the pigment epithelium Moreover spectroscopic methods demonstrated clearly that the yellow retinal pigment was different both from those of egg yolk (now known to be a mixture of xanthophyll and zeaxanthin) and from the corpus luteum of the cow (now known to be mainly β-carotene) Over a period of some 20 years extensive studies of the main properties of visual purple were carried out It was shown that the pigment may be held in aqueous solution by bile salts [11] and that it behaves as a colloid in failing to diffuse from such solutions through a semi permeable membrane It could be salted out with magnesium or ammonium sulphate [12] Either in the retina or in solution the 'purple' colour was lost by treatment with acetone, alcohol, chloroform, heavy metal chlorides alkalis or mineral acids [13] These properties of course, were indicative of a protein complex

The sensitivity of visual purple to light

The presence of visual purple in dark adapted retinas and its absence from retinas adapted to bright sunshine was another point established in these early experiments Kuhne [11] demonstrated that when retinas were taken from dark adapted frogs, and were exposed to sunshine, the deep red colour was changed to a bright orange, known as "visual yellow" This colour then faded slowly leaving the retina colourless after it had stood for about an hour at room temperature Of these two stages, however, only the first occurs under the influence of light, whereas the second is thermal Thus Wald [8] points out that if the retina is illuminated at 0° C the only change in colour is from red to yellow

Recognition of the sensitivity of visual purple to light supplied another clue to the understanding of night blindness, since it had long been realised

References p 276

that preliminary exposure to bright light was an important predisp factor Experience had shown, moreover, that poor dark vision might after a day of bright sunshine, particularly in snow covered montains dericn and Holm [14] made an important advance in linking together exp to bright light, defective visual purple formation and vitamin A defic in the etiology of night blindness Thus they were able to show that light adapted rats were placed in the dark the rate at which their re formed visual purple was reduced by deficiency of vitamin A Tansley [15] firmed that deprivation of vitamin A in rats reduced their efficiency in for rhodopsin

Vitamin A as a component of visual purple

So far it had been recognised that the fo tion of visual purple was influenced by min A, but there was no evidence to imp the vitamin directly in the visual processes It seemed quite probable the vitamin might improve dark adaptation merely by maintaining health of the whole body, including the retinal cells responsible fo formation of visual purple

The first step towards a realisation of the more immediate role of the min in vision came with proof of its actual presence in the retina In Holm [16] detected the vitamin in the retina of the calf, and two years Yudkin [17] followed with observations on pigs The evidence, judged on dern standards did not point to a very high concentration of the vita Thus Yudkin was content to state that in biological tests dried hog s r was a decidedly richer source than butter fat, which contains only a 20 i u of preformed vitamin A per g Although he also observed a colour when retinal extracts were treated with arsenic trichloride he r gave no proof that vitamin A was any more concentrated in the retina in many other parts of the body There was no need, therefore, to ass that the vitamin was directly concerned in dark adaptation If small amo are widely distributed why should the vitamin be excluded from the ret

Evidence of the direct participation of vitamin A in dark adaptation h ever was obtained in Wald's classical research In extensive studies on fr pigs, sheep and cattle he found vitamin A in the retinas and in the comb pigment epithelia and choroid layers [18] The vitamin was identified by absorptions at 328 mμ in the ultraviolet and at 620 mμ in the antimony chloride test and also by its anti-xerophthalmic and growth promo activity Proof of the participation of the vitamin in the changes accomp ing dark adaptation soon followed [19] Thus Wald found that the dark adap retina of the bullfrog *Rana catesbiana*, contained only a trace of vitami which could be extracted with benzine in the dark without injuring visual purple If the retinas were then exposed for a short time to light fur

extraction with benzine now produced a yellow pigment, which was named "retinene" The story was then completed by allowing retinas which had been bleached by light to stand for about an hour at 25° C, either in the dark or in the light The yellow colour seen immediately after light adaptation had now been lost, and extraction with benzine produced no retinene In its place substantial amounts of vitamin A had appeared (Fig 17)

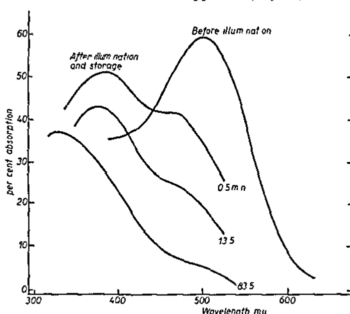


Fig 17 Conversion of retinene to vitamin A in a fresh aqueous extract of bullfrog retinas The original solution before illumination displayed the rhodopsin maximum at 502 mμ (right hand curve) The solution was then exposed to light for 25 seconds and was examined at further intervals after storage in the dark The curves on the left were observed after storage for 0 5 13 5 and 83 5 minutes respectively The spectroscopic evidence indicates that the rhodopsin first gave rise to an orange intermediate compound (max 480 mμ) and retinene (385 mμ) These were later replaced by vitamin A (330 mμ) The inflection at 500 mμ in the final curve may be ascribed to small amounts of re generated rhodopsin (after Wald)

From these findings Wald formulated a cycle, or triangle, as shown in Fig 18 We shall see later that this scheme has subsequently been slightly modified with the removal of the arrow at the left hand side of the triangle. We now know that vitamin A does not combine directly with protein to form

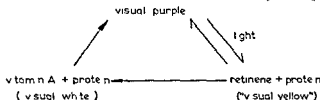


Fig 18 Wald s original cycle (1935) showing formation of visual purple from vitamin A and protein and its decomposition by light

References p 276

visual purple Moreover our conception of visual yellow has become more complicated There are probably several unstable complexes between retinene and protein These include "indicator yellow", which is only slightly coloured in neutral and alkaline solution but strongly yellow in acid solution In spite of these imperfections, however, Wald's 1935 cycle remains a landmark in the history of research on vitamin A

The Anatomy of the Eye

It may be helpful, before we go on to discuss the more recent discoveries on vitamin A in vision, to include a brief account of the anatomy of the eye Obviously a full description would be out of place, and we shall confine our attention to points relevant to the role of vitamin A in the retina (Fig 19)

The layers of the eye ball and retina If we work from the back of the eye ball forward towards the lens and pupil, avoiding the point of entry of the optic nerve, we start with the conjunctiva, which is a thin transparent mucous membrane, covering the white of the eye and also the inner side of [illegible]

[illegible]

connective tissue Although only about 0·5 mm thick, in humans, it is very strong, and when required can support high intra ocular pressure without stretching Going forward again we come to the choroid, a layer 0·1–0·2 mm thick, which has its main function in carrying blood vessels

We now arrive at the retina, and strike first into the pigment layer This is made up of epithelial cells which produce a pigment of the melanin type The next layer is perhaps rather surprising to the uninformed. It consists of the rods and cones, which act as photo-receptors, placed with their free ends directed back on to the surface of the pigment layer This appears to point them away from the source of light In the human eye there are some 7 million cones, which are concerned with acute vision in strong light and with the appreciation of colour The number of the rods is much greater, with between 70 and 140 millions.

We arrive next at a thin membrane limiting the region of the rods and cones and thence to a layer of nuclei belonging to the rods and cones These nuclei appear to be part of the body of the cones, but to be attached by fibrils to the rods The remaining layers, before we emerge into the vitreus humour are made up mainly of nerve cells Thus the nuclei of the photo-receptors communicate with so called ganglion cells near the surface of the retina through the medium of bipolar cells The junctures between the photo-

receptor nuclei and the outer end of the bipolar cells and between the inner end of the bipolar cells and the ganglions, are effected by the intertwining of very fine branches at the end of the nerve fibrils (Fig 19) From the ganglions nerve fibrils run over the surface of the retina to join up with the optic nerve, which proceeds out of the back of the eye on its way to the brain

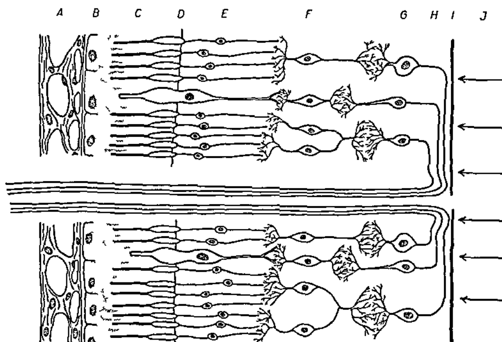


Fig 19 *Structure of the vertebrate retina* (A) Choroid with blood vessels (B) Pigment epithelium producing melanin pigment which can protect the outer limbs of the rods against intense illumination (C) Rods and cones Rhodopsin is present in the outer limbs of the rods which are shown black (D) Outer limiting membrane (E) Outer nuclear layer (F) Inner nuclear layer of bipolar cells (G) Nerve ganglion cells (H) Nerve fibres running over the surface of the retina and back into the optic nerve The point of exit of the nerve forms the slightly raised optic disk which is a blind spot (I) Inner limiting membrane (J) Direction of light rays

Several points of interest deserve mention (1) Before reaching the active ends of the photoreceptors the light rays, as will have been surmised, must traverse several layers of the retina concerned with converting the stimulus of light into nerve impulses The obstacle presented by these layers must obviously be much less formidable than we might imagine (2) The retina has no blood supply at all, and must receive its nutriment by diffusion from the choroid (3) The papilla, which marks the point of entry of the optic nerve and blood vessels, is a small protrusion, about 1 5 mm in diameter It is also known as the optic disk, and its examination by the ophthalmoscope may give information of value to the pathologist Thus papilloedema, with increased protusion of the disk is a sign of vitamin A deficiency, particularly in

References p 276

cattle (5) Owing to the absence of photoreceptors the papilla is a 'blind spot' Not far away, however, is a depressed area of about the same size, called the *macula lutea*, in which vision is more acute than in other parts of the retina Its central portion, the *fovea centralis*, contains *only cones* and these are less overlaid with nerve fibres than are the photoreceptors in other parts of the retina The fovea is used for purposes requiring sharp focussing of the eye in good illumination, as in reading

The reaction of the retina to strong and weak illumination

Histological comparisons of light adapted and dark adapted retinas have indicated that the cones are mainly concerned with vision in strong light, and the rods with vision in semi darkness Thus in strong light the narrow, free ends of the rods, which contain rhodopsin, are shielded by pigment protruded from the pigment layer In weak light, however, the rods are uncovered and the pigment covers the cones instead

This transfer of duties between the rods and cones is strikingly confirmed by the shape of typical dark adaptation curves, which relate the efficiency of vision in the dark with the time since strong illumination was stopped A distinct break is seen in the curves after the subject has been kept in the dark for some minutes This presumably corresponds to the covering of cones with pigment and the unmasking of the rods (Chap 29, Fig 25)

RECENT RESEARCH

The most recent additions to our knowledge of the role of vitamin A in vision have been made in three main stages Firstly there has been the vital clue, provided by Morton and his colleagues, that retinene is the aldehyde of vitamin A The observations which established this crucial point are described in Chapter 11 and need not be repeated here Secondly important studies have been made by Wald and others on the enzyme systems by which oxidations and reductions between vitamin A and retinene are effected Thirdly our story has been embellished by Wald s discovery of the role of *cis trans* isomerism in the preparation of retinene for its combination with opsin in the formation of visual purple

Vitamin A from bleached rhodopsin in cell free extracts

The first step towards the recognition of the importance of an enzyme system in the formation and decomposition of rhodopsin was made in attempts to obtain vitamin A from cell free solutions of rhodopsin Wald[20] prepared neutral rhodopsin solutions by extracting the retinas of various species with digitonin as a detergent and studied the products formed when they were bleached by light Various yellow pigments were detected but the final product was invariably retinene, as characterised by its absorption band at 664 mμ in the antimony tri

chloride test It was later reported by Bliss [21] that vitamin A was formed in large quantities by fresh bleached solutions of rhodopsin which had been obtained from unhardened retinas If the retinas had been hardened by alum, however, the solutions of rhodopsin subsequently obtained from them would not give vitamin A It appeared, therefore, that some enzymic factor was immobilised by the alum and so excluded from the extract

Wald and Hubbard [22] agreed that vitamin A was formed by rhodopsin solutions, but only if they were perfectly fresh If the solutions were stale the previous observation that the bleaching stopped at retinene was repeated Some essential factor was therefore lost on storage Confirmation that intact cells were not necessary for the production of vitamin A was obtained in experiments with aqueous suspensions of ground up powder obtained from dried cattle retinas This material also contained the factor which was necessary for the production of retinene

Incomplete enzyme system in rod outer limbs

Wald and Hubbard were prepared to undertake a fractionation of the retinal enzyme systems by chemical means but instead were helped by a natural division of the enzyme constituents on an anatomical basis During dissections of the retina it had often been noticed that the outer links of the rods which are the narrower free end (shown in black in Fig 19) became broken off at their point of attachment to the wider inner segments (shown in white in Fig 19) The outer limbs contain all the rhodopsin in the retina and their ready detachment from other tissues had in fact already been put to advantage by Lythgoe [23] and Saito [24]. Thus by the careful scraping of retinas dense suspensions of outer limbs could be obtained and used for the preparation of partially purified solutions of rhodopsin

In studying the products of bleaching the rhodopsin of the rod outer limbs separated from other constituents of the retina, Wald and Hubbard found that the process stopped at retinene A similar result was obtained if the limbs were left in the same vessel with the remainder of the retina which had been kept in more or less intact condition If the tissues of the retina were broken by grinding, however, a factor was liberated which caused retinene to be reduced to vitamin A Thus a suspension of rod outer limbs together with a clear, colourless water extract of retinas constituted a complete system for the reduction of retinene

The role of cozymase

The next finding was that the retinal extract could be dispensed with and replaced by a boiled muscle extract Wald and Hubbard inferred that the factor supplied by the meat must be cozymase (diphosphopyridine nucleotide, DPN) which assists many apoenzymes concerned in hydrogen transfer A pure preparation of DPN was

References p 276

tried first, and found inactive It became active, however, after reduction to the dihydro compound by treatment with sodium hydrosulphite Alternatively fructose diphosphate could be added to the system, which allowed the reduction of DPN to $DPNH_2$ by enzymic means

At this stage, therefore, the production of vitamin A from rhodopsin could be demonstrated in a system consisting of (1) rhodopsin (2) an apoenzyme, retinene reductase (3) DPN and (4) fructose diphosphate Of these constituents (1) and (2) were present in the rod outer limbs Presumably the limbs also contributed an enzyme which catalysed the reduction of DPN to $DPNH_2$ Wald pointed out that the introduction into the system of DPN, which contained nicotinamide as its prosthetic group provided an interesting example of interaction between vitamins

The enzymic oxidation of vitamin A to retinene

So far Wald had attempted to reproduce, in simplified systems, the changes which occur in the retina after bleaching We must now turn to attempts to study the reverse process, in which rhodopsin is built up from vitamin A and protein It was soon realised, for reasons which will appear later, that the first step in this process must be the dehydrogenation of vitamin A to retinene

Bliss [25], doubtless inspired by Wald's discovery of the importance of cozymase, had the idea that the conversion might be promoted by the well known enzyme alcohol dehydrogenase He therefore obtained a concentrate of this enzyme from liver, and tested it in a system which also contained DPN and an aqueous dispersal of vitamin A stabilised by Tween 80 Bisulphite or cyanide was also added to trap the aldehyde as it was formed, and so displace the equilibrium between vitamin A and retinene in the direction required Under these conditions up to 40% of added vitamin A could be converted to retinene Moreover by replacing the DPN by $DPNH_2$ retinene could be changed back to vitamin A

The regeneration of rhodopsin in vitro

Let us now turn to the resynthesis of rhodopsin in the dark after bleaching in light, either in the retina or in solution Kuhne [26] had found that partial resynthesis occurred provided that illumination was stopped as soon as the pigment had turned yellow, and had not been continued to the point of complete discolouration Hecht *et al* [27] and Chase and Smith [28] confirmed the regeneration of rhodopsin in solution, but obtained recoveries of only about 15%

Wald and Brown [29] concluded that the amount of rhodopsin which is regenerated decreases with the severity and time of the bleaching process Thus if the changes in rhodopsin are stopped in their early stages, either by extreme cold or by making up the rhodopsin in a dried gelatine film, the

recovery in the dark may be as much as 50% On the other hand the regeneration in a solution which has been illuminated for an hour, at room temperature may be very low indeed

Attempts were made to obtain clues as to the mechanism of the formation of rhodopsin by adding various components in the reaction from various sources By adding synthetic retinene to a rhodopsin solution before bleaching it was found that 70% regeneration could be obtained

Regeneration of rhodopsin in the presence of vitamin A, however, was much less efficient [30] Thus aqueous retinal extracts, supplemented with vitamin A and cozymase gave only 10% regeneration From this evidence, and from the results of many other experiments, it was concluded that vitamin A is converted to retinene before it is used for the formation of rhodopsin

With all this added information Wald [31] in 1951 revised his scheme for the formation of rhodopsin as shown in Fig 20 With Hubbard [30] he had already shown that an effective model system could be set up with "purified" opsin from cattle retinas vitamin A from fish liver oil, crystalline alcohol dehydrogenase from liver, and cozymase, presumably in its reduced form

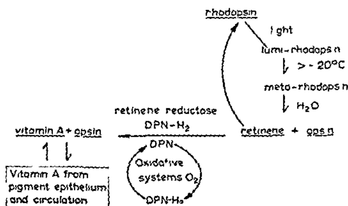


Fig 20 Wald s 1951 rhodopsin system which is now known to operate in conjunction with the *cis trans* changes shown in Fig 21

Cis trans *isomerism of retinene in rhodopsin formation* Apart from our ignorance of the nature of opsin it seemed at this point that the end of a chapter of research had been reached, with the successful solution of many complicated problems There remained a further twist in the story, however, to test Wald's ingenuity and technical skill We have just mentioned that a model system could be made to form rhodopsin from vitamin A isolated from fish-liver oil To Wald s surprise the same system would not work when pure synthetic vitamin A was substituted for the natural form [32]

Cis-trans isomerism appeared to be the probable explanation It was re-

References p 276

called that Chase [23, 24] had long ago reported that rhodopsin could be regenerated when it was bleached by blue light, but not when it was bleached by yellow light Light promotes *cis trans* isomerisation, and Wald inferred that after liberation from rhodopsin retinene was isomerised by blue light, which it absorbs Yellow light, which is not absorbed, did not cause isomerisation In confirmation of this difference it was found that the absorption maximum for retinene produced by blue light was displaced 5 mμ towards the shorter wavelengths as compared with retinene produced by yellow light

Hubbard and Wald [35] confirmed the significance of *cis-trans* isomerism by showing that all-*trans* retinene would not combine with opsin to form rhodopsin Combination took place, however, after the retinene had been submitted to treatment calculated to cause isomerisation, such as illumination in the presence of a trace of iodine After intensive tests on various isomers of retinene successful formation of rhodopsin was obtained with a 2,4 di *cis* form designated neoretinene *b*

It will have been noted that the retinene liberated from rhodopsin by yellow light, which does not cause isomerisation, has the absorption spectrum of all-*trans* retinene Wald concludes, therefore, that retinene is combined into rhodopsin in one isomeric form, but is liberated from rhodopsin in a different form A cycle of isomerisation, as shown in Fig 21, is therefore an intrinsic component of the rhodopsin system

In the body all-*trans* vitamin A is probably easily isomerised There would seem no point, therefore, in administering *cis* isomers with the idea of increasing the efficiency of dark adaptation

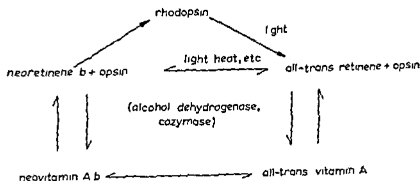


Fig 21 Wald s cycle for the isomerisation of vitamin A and retinene during the formation of rhodopsin

Properties of rhodopsin In spite of our knowledge of the factors influencing the formation and decomposition of rhodopsin our information on its chemical nature remains slight As Wald [1] has pointed out it cannot be obtained in simple aqueous solution, but has to be stabilised by

a suitable detergent This results in the formation of micelles, and disallows determination of the molecular weight

The absorption bands of rhodopsin are at 500, 350 and 278 mμ The band at 500 mμ is associated with the red colour, and with the function of the whole molecule as a photoreceptor The shape of this band agrees closely with the sensitivity of the retina to light of corresponding wavelength The band at 350 mμ is associated with retinene, and that at 278 mμ with opsin Even when opsin is disunited from retinene a band at 278 mμ is still shown, and is indicative of nothing more specific than a protein which contains amino acids having aromatic groups

Wald considers that sulphydryl groups play a part in linking retinene to opsin

Other visual pigments The whole story which has been told for rhodopsin could be repeated for porphyropsin, the main visual pigment in fresh water fishes which is derived from vitamin A_2 (Chap 11) The absorption bands of porphyropsin are at 522 378 and 278 mμ Vitamin A_2 can be dehydrogenated by the same enzyme systems as will dehydrogenate vitamin A_2 Retinene$_2$ (Chap 11) will combine with opsin which has been optained by the bleaching of rhodopsin to form porphyropsin

Wald [36] has reported the detection of a further pigment, iodopsin, in chicken retinas It absorbs at 565 mμ and is bleached by red light Presumably it acts as a photoreceptor for the cones

Various other visual pigments not necessarily related to vitamin A, have been reported by Granit [37], von Studnitz [38] and others Discussion of the reality and significance of these pigments seems beyond the scope of this book.

In 1938 Adler and von Euler [39] were impressed by the large amounts of riboflavin in the eyes of certain fishes The possibility that this yellow, fluorescent pigment can have some unsuspected role in vision must not be too lightly dismissed

Opsin as a key to photoreception As a closing thought to this chapter we may reflect on the vital role of the protein opsin in diverting vitamin A from its normal paths of metabolism, and so making scotopic vision possible Thus it has been clearly proved that the liver contains all the enzyme components necessary for interconversions between vitamin A and retinene The same system must also be present in the intestinal walls, where retinene may readily be reduced to vitamin A Apparently only the retina contains, in opsin, an aldehyde trapping agent which is capable of stabilising retinene, and so of diverting vitamin A from its normal systemic metabolism into a highly specialised side system

References p 276

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CHAPTER 23

Biological Activity of Congeners of Vitamin A

In the preceding chapter, on vitamin A in vision, we have seen a good example of the concentration of research on a narrow and highly specialised field We must next turn our attention to the biological activity of natural and artificial congeners of vitamin A On a chemical basis our field is now much wider, and the concentration of research has been correspondly reduced Adequate investigations have only been made on those aspects of the subject which appear to have special importance For the rest it is often necessary to draw conclusions from the results of a few isolated tests, which have not seemed worth the trouble of confirmation by other workers

It will be most convenient first to deal briefly with the general problem of the effect of chemical structure on vitamin A activity Our review will cover both natural and artificial derivatives and congeners, some active and some inactive We shall then go on to discuss more fully those congeners about which detailed knowledge is available, and which justify special discussion by reason of their biological or theoretical importance Thus the neo forms of vitamin A, vitamin A aldehyde and vitamin A_2 will be discussed as important natural derivatives of the vitamin, and vitamin A acid as an artificial derivative which nevertheless has greater theoretical interest. A final section will deal with claims, of rather doubtful authenticity that vitamin A may exist in certain sources in hidden forms which cannot be detected by the usual chemical and physical methods

The biological activity of different provitamins, which has been covered in Chapters 8 and 15, will not generally be discussed further in this chapter An exception will be made, however, for astaxanthin which may perhaps be considered as relevant to the question of hidden forms of vitamin A For the chemistry of the congeners of vitamin A reference may be made to Chapter 11

Structural Features Influencing Vitamin A Activity

Several excellent tables giving information on the biological activities of congeners of vitamin A have been included in a review by Isler [1] The help of

References p 289

this review in correlating our knowledge in this field is gratefully acknowledged by the present author.

Cis-trans isomerism Biological activity is retained, but may be reduced to 23–75% of that of the all-*trans* form, according to the configuration of the isomer (see below)

Esterification Apart from minor differences in the efficiency of absorption, which have been mentioned in Chapter 18, esters of vitamin A have substantially the same biological activity as the free alcohol, molecule for molecule This finding applies both for natural and artificial esters Thus Hamano [2] found that after esterification to the β-naphthoate or anthraquinone-β-carboxylate the vitamin retained its full activity Robeson and Baxter [3] reached the same conclusion for the acetate, succinate, palmitate and *p*-phenylazobenzoate, and Isler *et al* [4] for the butyrate, laurate, stearate, oleate and benzoate

Ether formation Vitamin A alkyl and aryl ethers are of interest as substances not known to occur naturally, but readily obtainable by synthesis Hanze *et al* [5] found that the methyl ether was about equal to the free alcohol in biological activity The phenyl ether, however, was found by Isler *et al* [4] to have only about one-tenth of the expected activity

Oxidation products Conversion of vitamin A to its aldehyde does not effect its biological activity Vitamin A acid is also highly active under certain conditions of administration Both these derivatives will be discussed in greater detail later

In other oxidation products the activity of the vitamin may be much reduced or lost Hepaxanthin or vitamin A epoxide, was reported by von Euler, Karrer and Zubrys [6] to have little, if any, biological activity

Hydrogenation The saturation of one or more of the unsaturated linkages causes complete loss of activity Thus dihydro-vitamin A was found inactive by Gould [7], tetrahydro-vitamin A by Ruzicka and Fischer [8] and perhydro-vitamin A by Karrer and Morf [9]

Dehydrogenation The presence of an extra unsaturated linkage, as in vitamin A_2, causes reduced biological activity, at least when the estimations are made with rats Vitamin A_2 will be discussed later in greater detail

Loss of oxygen Axerophthene, which differs from vitamin A in having no hydroxyl group, has about one-tenth of the activity of the vitamin [10, 11]

Dehydration Anhydrovitamin A, another artificial product, has only very low biological activity Thus Shantz *et al* [12] found that two specimens had each about 0·4% of the activity of the vitamin

Ketone formation According to Arens and Van Dorp [13] the C_{21} ketone corresponding to vitamin A acid has about one tenth of the activity of the vitamin

Condensation Although the structure of kitol is not known with certainty it seems reasonable to regard this C_{40} derivative of vitamin A as a condensation product We have seen in Chapter 11 that it is unique among vitamin A derivatives in having no biological activity itself, but in forming vitamin A when heated to over 200° C

Changes in the carbon skeleton Milas *et al* [14] found that the extension of the side chain of vitamin A by one extra carbon atom greatly decreases the biological activity Thus homovitamin A ethyl ether was found to have only 1 5% of the activity of the vitamin

The omission of a methyl group from the side chain as in norvitamin A methyl ether was found by Cheeseman *et al* [15] to reduce the activity to 3% of that of the unchanged vitamin Various C_{16} C_{17} or C_{19} acids were reported by Heilbron and his colleagues [16 19] to have about 0 1% of the activity of vitamin A

General conclusions Before generalisations are attempted two important reservations must be made Firstly it is obvious that many estimations have in fact been only approximately correct A preliminary statement that a derivative of the vitamin retains its full activity may well overlook a decrease in potency of 20 or 30% An estimate that a derivative has 1% of the activity of the vitamin may be based only a few tests which indicated slight responses to high doses Secondly the tests

TABLE 34

EFFECT OF VARIOUS CHEMICAL CHANGES ON THE BIOLOGICAL ACTIVITY OF VITAMIN A

Process	*Product*	*Approximate activity All trans vitamin A = 100*
Esterification	Natural or artificial esters	100
Oxidation	Aldehyde	100
Cis isomerism	*Cis* isomers	23–75
Ether formation	Phenyl or methyl ethers	10–100
Dehydrogenation	Vitamin A_2	30
Loss of oxygen	Axerophthene	10
Ketone formation	C_{21} ketone	10
Demethylation	Norvitamin A	3
Addition CH_2	Homovitamin A	1 5
Dehydration	Anhydrovitamin A	0 4
Condensation	Kitol	0
Oxidation	Epoxide	0
Hydrogenation	Dihydro vitamin A etc	0

usually adopted measure the ability of the substances under investigation to restore growth in deficient rats. It seems questionable whether the ability to restore growth, sometimes only temporarily, must always imply that the substance can replace the vitamin in all its functions As we shall see later it is highly probable that growth promotion may sometimes be divorced from the ability to participate in the mechanism of scotopic vision

With these reservations in mind, however, the factors affecting biological activity may be graded roughly as in Table 34

Cis-trans Isomerism

In their report of the isolation of neovitamin A Robeson and Baxter [20] stated that the biological activity of this 2 *cis* isomer was substantially equal to that of the all-*trans* form This conclusion was not surprising since the two isomers were shown to be intra-convertible in the animal body Thus when depleted rats were given substantial doses of neovitamin A the proportions of the two isomers subsequently recovered from the liver were 18% of neovitamin A and 82% of all-*trans* vitamin A. When all-*trans* vitamin A was administered the liver oil contained 11% of the neo form and 89% of unchanged all-*trans*. With either isomer as the starting point, therefore, the all *trans* form predominated in the final mixture

Careful experiments by Harris, Ames and Brinkman [21] necessitated only slight modifications of the original findings On a molar basis neovitamin A was found in growth tests with rats, to have only 81% of the activity of all-*trans* vitamin A But since $E^{1\%}_{1\,cm}$ at 328 mμ is slightly lower for neovitamin A than for all *trans* vitamin A the activities on an E basis were as 85% to 100% On the basis of liver storage tests, again with rats, the neo form had 72% and 76% of the activity of the all-*trans* form by the two methods of calculation

More recent tests by Ames, Swanson and Harris [22] have included assays not only on the familiar neo isomer but also on other isomers, which were obtained by synthesis The results were as follows

*Isomer tested**	*Percentage of activity of all* trans *vitamin A*
2-Mono-*cis*-vitamin A acetate (neo)	75
6 Mono *cis* ,, ,,	22
2 6-Di *cis* ,, ,,	24
2 4-Di *cis* ,, ,,	23

The influence of isomerism on the activity of vitamin A aldehyde will be discussed below

* Geneva system of numbering

ALDEHYDE FORMATION

The conversion of retinene to vitamin A

The preservation of biological activity in vitamin A aldehyde may be explained by the ease of its reduction in the body to vitamin A alcohol Glover Goodwin and Morton[23] found that when retinene was given orally to rats part of the dose could be recovered unchanged from the contents of the stomach and small intestine during the following 4–24 hours The walls of the intestines and the liver however were found to contain vitamin A mainly in the esterified form without any retinene They found moreover that the ability to reduce the aldehyde was not confined to the intestines Thus in rats which were killed 3–7 weeks after subcutaneous injections of retinene substantial amounts of vitamin A again mainly esterified could be demonstrated both in the tissues near the site of the injection and also in the liver Gounelle *et al* [24] observed that the vitamin A level was increased in the blood plasma of human subjects six hours after substantial doses of retinene had been given

The biological activity of retinene and its isomers

Ames Swanson and Harris[25] compared various retinene isomers with all *trans* vitamin A acetate in biological tests The following results were obtained

*Retinene isomer**	*Percentage of potency of all* trans *vitamin A (on molar basis)*
All *trans*	91
2 Mono *cis* (neo)	93
6 Mono *cis*	19
2 6 Di *cis*	17
2 4 Di *cis*	48

It will be recalled that the activity of 2 4 di *cis* vitamin A had been found to be only 23% of the activity of all *trans* vitamin A It is interesting therefore that the aldehyde with the 2 4 di *cis* configuration is about twice as potent as the corresponding alcohol Ames and his colleagues suggest that in some instances isomerisation to the all *trans* configuration takes place *in vivo* more easily in vitamin A aldehydes than in the corresponding alcohols The same superiority of the aldehyde but with a much smaller difference was found between the 2 *cis* (neo) compounds As a side issue the α ionone analogue of retinene was tested It had only 2% of the potency of vitamin A acetate Nevertheless evidence was obtained of its reduction to α vitamin A which was stored in the liver

* Geneva numbering

References p 289

Vitamin A Acid

The biological activity of vitamin A acid raises several interesting questions which would repay further research. Thus the activity seems to depend greatly on the method of dosing. Irrespective of the dosing technique how ever, we have [illegible]
Even after dos [illegible]
the activity se[illegible] exerted without preliminary conversion to vitamin A alcohol, as has been found for retinene

The effect of the method of dosing

Arens and Van Dorp [26] first stated that vitamin A acid had about one tenth of the activity of vitamin A when administered to rats in the conventional manner in solution in arachis oil When injections of the sodium salt were made, however, the activity was increased to about half that of vitamin A Later it was found that even better responses were obtained by administering the sodium salt orally, which gave an activity equal to that of the vitamin [27]

Vitamin A acid	*Percentage of activity of vitamin A claimed*
Dissolved in arachis oil	10
Sodium salt by injection	50
Sodium salt orally	100

No reduction to vitamin A

From past experience it was to be expected that the intense activity of vitamin A acid must indicate that it could undergo reduction in the body to vitamin A alcohol To check this point Arens and Van Dorp [28] gave massive subcutaneous or oral doses of the sodium salt to depleted rats Even doses of 10 mg did not cause the appearance of vitamin A in the liver

In the author's laboratory Sharman [29] confirmed that the sodium salt of vitamin A acid has intense growth-promoting power when given, either orally or by injection, to depleted rats The acid, however, was always less potent than carotene given orally in oily solution Thus the activity was less than half of that of vitamin A No storage of vitamin A was found in the liver even after massive dosing It was difficult even to find the unchanged acid in the body or intestines, but in one experiment about 6% of the dose, as measured by the absorption band at 343 $m\mu$, was recovered from the stomach contents Harris [30] has found that vitamin A acid is poorly absorbed from the intestines

There is agreement, therefore, on the growth promoting power of vitamin A acid, and on its failure to undergo conversion *in vivo* to vitamin A alcohol For the present it is difficult to see how vitamin A acid could replace vitamin

A in its visual functions An ability to undergo reduction to retinene would also imply the ability to form vitamin A alcohol since the aldehyde is so readily reduced The author has suggested that a metabolic watershed exists between vitamin A aldehyde and vitamin A acid [31] Thus the aldehyde is reduced back to vitamin A alcohol but the acid is presumably further oxidised to unknown products It seems probable that the acid can fulfil some of the functions of vitamin A in the general system but presumably not in the retina

VITAMIN A_2

The predominance of vitamin A_2 over vitamin A_1 in some fresh water fishes was mentioned in Chapter 13 There is no evidence that vitamin A_2 can be produced in species other than fishes except after experimental dosing with retinene$_2$ It also seems clear that vitamin A_1 and vitamin A_2 are not readily intraconvertible even in fresh water fishes In spite of being a foreign sub stance to the mammal however vitamin A_2 has substantial activity in growth tests on rats

Biological activity The interpretation of early tests on concentrates of vitamin A_2 was made difficult by the presence of vita min A_1 as impurity Tests on a virtually pure preparation of natural vitamin A isolated by Shantz [32] however indicated 40% of the activity of vitamin A_1 A synthetic preparation of all *trans* vitamin A_2 was found by Farrar *et al* [33] to have 30% of the activity of vitamin A_1

Gillam *et al* [34] hinted that some liver oils of fresh water fish may be toxic Jensen *et al* [35] reported that massive doses of vitamin A_2 were less well tolerated by rats than equal doses of vitamin A_1

Replacement of vitamin A_1 by vitamin A_2 in the rat The inability of vitamin A_2 to be hydrogenated *in vivo* to vitamin A_1 has been proved in experiments by Shantz *et al* [36] It was first established by means of spectrophotometric readings in the antimony tri chloride test that the livers blood serum and light adapted retinas of nor mal rats contain only vitamin A_1 and no vitamin A_2 In the same sites in rats which had been given a deficient diet much smaller amounts of vitamin A_1 were found Dosing of such animals for some weeks with 100 units of vita min A_2 daily caused restoration of growth but the amounts of vitamin A_1 in the liver and blood were not increased Substantial amounts of vitamin A_2 however were superimposed upon the pre existing traces of vitamin A_1 which were retained tenaciously In extracts of the dark adapted retinas made with sodium glycocholate the absorption band of porphyropsin at 520 mμ was observed and not the band at 500 mμ characteristic of rhodop sin It appears therefore that the rat is able to employ a form of vitamin A

References p 289

which is foreign to its species but is not able to convert this form to the vitamin typical of its own species

Cama *et al* [37] demonstrated that retinene$_2$ can cure xerophthalmia and restore growth in rats deficient in vitamin A After heavy dosing with the aldehyde vitamin A_2 could be detected in the liver

According to Gillam [38] vitamin A_2 accompanies vitamin A_1 in the livers of the seal and otter, which eat fish A similar observation was made on the liver of the carnivorous giant monitor (*Varanus salvator*).

Replacement of vitamin A_2 by vitamin A_1 in fresh water fish

Since vitamin A_2 is characteristic of fresh water fishes it might be surmised that these species must possess the power to dehydrogenate vitamin A_1 with the introduction of the required extra double bond Morcos and Salah [39], however, failed to demonstrate that vitamin A_2 can be derived from vitamin A_1 by two fresh water fishes from the Nile, *Clarius lazera* and *Tilapia nilotica* When massive oral or subcutaneous doses of vitamin A_1 were given to these fishes there was no increase in the amount of vitamin A_2 in their livers, although this form normally predominates in these species The vitamin A_1 contents of the livers, however, were substantially increased Some of the fish were kept under observation for a month after dosing with vitamin A had been stopped, but no ill after-effects were observed

The biological properties of vitamin A_2 are compared with those of other congeners of vitamin A_1 in Table 35

TABLE 35

CONTRASTS IN THE BIOLOGICAL ACTIVITIES AND METABOLISM OF CERTAIN CONGENERS OF VITAMIN A

Substance	*Grading of activity*	*Reaction in body*
Vitamin A aldehyde	+++	Readily reduced to vitamin A
neovitamin A	++	Mostly isomerised to all *trans* vitamin A
Vitamin A acid	++	Disappears *Not* reduced to vitamin A
Vitamin A_2	+	Absorbed into body unchanged Not converted to vitamin A_1
Vitamin A epoxide	0	Fate unknown Possibly the epoxide is a metabolic product of vitamin A *in vivo*

Alleged Hidden Forms of Vitamin A

Finally we must deal with rumours that sources of vitamin A activity may exist in which it is impossible to detect the vitamin or any of its known pro vitamins or congeners by the accepted physical or chemical methods Recent research on the so called lard factor however has suggested that this field abounds with pitfalls for the unwary The greatest caution must therefore be exercised both in the acceptance and interpretation of evidence

For many years workers on vitamin A deficiency in rats have been puzzled

[40 41] It has also been observed that there is a wide gap between the daily dose of vitamin which is necessary to allow good growth and the dose which is necessary to cause the accumulation of reserves of vitamin in the liver [42 43] Moreover when single doses of vitamin A are administered to depleted rats it seems necessary for at least 100 i u to disappear before measurable storage commences [43 44] All these findings might suggest that the vitamin is em ployed to form some substance which is immediately responsible for promot ing growth and preventing xerophthalmia During dietary deficiency the health of the animal can be maintained in the absence of the vitamin itself *until supplies of this unidentified substance have been expended* When a deficient animal is dosed with vitamin A storage cannot take place in the liver until an adequate stock of the unidentified substance has been made

Obviously much of the above evidence should be reviewed in the light of the finding that vitamin A tends to go to the kidneys rather than to the liver at low levels of dosing [45] There remains another reason however for supposing that the vitamin may exert its action through an unknown inter mediary substance Thus in some of the sites which are particularly vulner

min A however a warning must be repeated against the uncritical accept ance of all the claims which have been put forward

Rats blood Evidence of a hidden form of vitamin A in the blood of albino rats was reported by Le Gallic [46] Groups of albino rats were started at intervals upon a diet deficient in vitamin A which contained muscle peptone dried yeast dextrin heated arachis oil minerals and vita min D When the animals had ceased to grow blood was collected from

References p 89

another group of rats which had been started upon the diet later, and which were still growing Chemical tests failed to reveal either preformed vitamin A, or carotene, in this blood When it was given to the first rats however, either orally or by injection, a prolonged restoration in growth was induced The same hidden vitamin A activity in the whole blood of growing animals was also found in prophylactic experiments

In later experiments Le Gallic [47] was unable to repeat his findings with piebald rats, but he claimed that deficient piebald rats could be cured by the blood of growing albino rats Sharman and Moore [48] tried to repeat the observations of Le Gallic on albino rats without success

The lard factor Studies on the vitamin A activity of lard have been long, and complicated In 1934 Randoin and Netter [49] reported that while neither casein nor lard was capable of supporting growth in rats, when given singly, "they were effective in combination" Thus when they were mixed together in appropriate proportions with dextrin, dried yeast and minerals, they could sustain life in rats for 6–12 months In 1948 Le Gallic [47], another French worker, supported this view in claiming that rats which had been restricted to his muscle peptone diet gave at least a temporary growth response after being transferred to a diet containing casein and lard Moreover livers taken from rats given casein and lard contained a hidden form of vitamin A, as claimed for blood (see above) Livers taken from rats given the muscle peptone diet were inactive

Randoin and Le Gallic [50] reviewed the early and more recent work and concluded that vitamin A and carotene act through the formation of a "hormonal factor A", presumably identical with Le Gallic's "blood factor",

with vitamin A, but the vitamin was essential for rats given an incorrectly balanced diet containing muscle peptone and arachis oil

In further studies Le Gallic [51] found that his rats stopped growing when given a diet containing lard and peptone, but resumed growth temporarily when the peptone was replaced by casein In mice a similar response was obtained by replacing the peptone by either casein or egg albumin In subsequent experiments however, carefully dried cod muscle was found to be superior to either casein or egg albumin in supplementing the action of lard [52]

We must now turn to the American side of the story, which was developed independently by Kaunitz and Slanetz [53] These workers subjected lard to vacuum distillation, and collected a volatile fraction containing 7% of the original material Chemical and spectrophotometric examinations indicated that not more than traces of vitamin A were present in this distillate, but its activity as a source of the vitamin to deficient rats was proved beyond doubt.

Later it was found that factors present in the distillate shared with vitamin A the power to protect rats against the ill effects of rancid lard The new factor was thought to differ from vitamin A however in being more resistant to destruction by rancid fat [54]

As already hinted the results of recent research on the lard factor have been rather disappointing At first the existence of the hidden factor was supported [55] Lard distillate was found to have a biological potency of about 10 i u per g but no vitamin or provitamin could be detected by spectro photometric or colorimetric methods A note of warning however was sounded by Herb Riemenschneider Kaunitz and Slanetz [56] who succeeded in detecting traces of vitamin A by chemical means in two lard distillates While the existence of hidden activity still seems possible it was obvious that most of the activity of the distillates was due to ordinary vitamin A Adequate confirmation by Ames and Harris [57] of the presence of small amounts of vitamin A in lard soon followed Thus distillates from eight specimens of lard prepared in specially cleaned stills contained 6 22 i u of vitamin A per g as measured by the antimony trichloride reaction These values corresponded to 0 6–2 5 i u per g in the original lard

The work of Lowe and Morton [55] may perhaps leave a lingering doubt whether the biological activity of lard can always be ascribed to the presence of traces of vitamin A It seems clear however that these small amounts of vitamin which are concentrated in the distillate provide in most instances a sufficient explanation of the observed activity

Vitamin A oxidised in food We have seen that when vitamin A is oxidised to its aldehyde biological activity is preserved but that when it is oxidised to the epoxide the activity is completely lost The possibility of biological potency surviving oxidation in foodstuffs may therefore depend on the direction taken by the oxidation Duboulez *et al* [58] have claimed that unknown forms of vitamin A are produced when foods

chemical and spectroscopic examinations

Astaxanthin in shrimps and copepods Fascinating studies on the vitamin A activity of extracts made from shrimps and prawns have given rise to a strong suspicion that astaxanthin may replace carotene as the main provitamin in these species Since astaxanthin is generally regarded as biologically inactive it may be discussed in this chapter as a possible source of hidden activity

In Algeria Grangaud and his colleagues [59 62] examined oils prepared from *Aristeomorpha foliacea* (*Penaeidae*) and *Aristeus antennatus* The oil from specimens caught during summer was coloured red by astaxanthin but

References p 89

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Later it was found that factors present in the distillate shared with vitamin A the power to protect rats against the ill effects of rancid lard. The new factor was thought to differ from vitamin A, however, in being more resistant to destruction by rancid fat [54].

As already hinted the results of recent research on the "lard factor" have been rather disappointing. At first the existence of the hidden factor was supported [55]. Lard distillate was found to have a biological potency of about 10 i.u. per g but no vitamin or provitamin could be detected by spectrophotometric or colorimetric methods. A note of warning, however, was sounded by Herb, Riemenschneider, Kaunitz and Slanetz [56] who succeeded in detecting traces of vitamin A by chemical means in two lard distillates. While the existence of hidden activity still seems possible, it was obvious that most of the activity of the distillates was due to ordinary vitamin A. Adequate confirmation by Ames and Harris [57] of the presence of small amounts of vitamin A in lard soon followed. Thus distillates from eight specimens of lard prepared in specially cleaned stills, contained 6–22 i.u. of vitamin A per g, as measured by the antimony trichloride reaction. These values corresponded to 0.6–2.5 i.u. per g in the original lard.

The work of Lowe and Morton [55] may perhaps leave a lingering doubt whether the biological activity of lard can always be ascribed to the presence of traces of vitamin A. It seems clear, however, that these small amounts of vitamin which are concentrated in the distillate, provide in most instances a sufficient explanation of the observed activity.

Vitamin A oxidised in food We have seen that when vitamin A is oxidised to its aldehyde biological activity is preserved, but that when it is oxidised to the epoxide the activity is completely lost. The possibility of biological potency surviving oxidation in foodstuffs may therefore depend on the direction taken by the oxidation. Duboulez *et al.* [58] have claimed that unknown forms of vitamin A are produced when foods rich in the vitamin are stored. Thus after storage such foods retain slight biological activity, even when vitamin A can no longer be detected by careful chemical and spectroscopic examinations.

Astaxanthin in shrimps and copepods Fascinating studies on the vitamin A activity of extracts made from shrimps and prawns have given rise to a strong suspicion that astaxanthin may replace carotene as the main provitamin in these species. Since astaxanthin is generally regarded as biologically inactive it may be discussed in this chapter as a possible source of "hidden" activity.

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References p. 289

virtually no carotene or vitamin A was present In biological tests with rats it was highly active in curing xerophthalmia but much less active in promoting growth Chromatographic and spectroscopic examinations indicated maxima at 305 and 317 mμ Oil from specimens caught during winter was much less intensely coloured and did not cure xerophthalmia Astaxanthin isolated from the summer oil without saponification was effective against xerophthalmia but astacene obtained as an artifact after saponification was ineffective

The extensive findings of Kon and his colleagues [63] [64] have differed from those of Grangaud in the detection of substantial amounts of preformed vitamin A in the same varieties of shrimps Since the vitamin is mainly present in the eyes which were presumably discarded by the French workers this difference is perhaps understandable In spite of the detection of vitamin A however Kon [65] seems convinced that astaxanthin must serve as a provitamin in shrimps Thus in *Meganyctiphanes norvegica* it appears that the amounts of carotene present in the diet are too small to account for the amounts of vitamin A accumulated in the eyes

Investigations by Lane [66] in Florida also appear to be relevant to the topics studied by Grangaud and Kon Oil was obtained from the small shrimp like celanoid copepods *Temora turbinata* and *Centropages typicus* and was found to be rich in total carotenoids but devoid of carotene When the oil was fed to the small fish *Limanda ferruginea* however it appeared to be much more effective than an allowance of carotene of the same yellow colour in giving rise to the storage of vitamin A in the liver Chromatographic adsorption of the copepod oil gave fractions absorbing at 430 and 310 mμ respectively When the fraction absorbing at 310 mμ was incubated with pulped caecal tissues from the *Limanda* the intensity of the antimony trichloride reaction for vitamin A was much increased

Goodwin s evidence of the conversion of carotene to astaxanthin in the locust has been discussed in Chapter 13 Proof that a change is also possible in the reverse direction giving carotene or vitamin A from astaxanthin would greatly simplify the story of vitamin A in the marine world

Vitamin A acid Taken as a whole our evidence on hidden forms of vitamin A may rightly be considered as fragmentary and inconclusive To avoid the danger of excessive scepticism however we may remind ourselves again of vitamin A acid (Food freshly fortified by the addition of the sodium salt of this potent synthetic substance would certainly have high biological activity but the additive would escape detection by the usual chemical and spectroscopic methods Although we may doubt whether hidden forms of vitamin A exist in natural systems therefore we have at least one example of a hidden form of vitamin being possible under artificial conditions)

A list of the alleged hidden sources of vitamin A is given in Table 36

TABLE 36

ALLEGED HIDDEN FORMS OF VITAMIN A

Description	*Investigator*	*Properties claimed*	*Comments*
Rat blood factor	Le Gallic	Promotes growth in other rats	Attempted confirmation failed
Lard factor	Kaunitz	Promotes growth Resists rancidity	Probably traces of vitamin A
Lard casein factors	Random	When together promote growth by giving the correct balance of nutrients The need for vitamin A is thus avoided	Probably traces of vitamin A in lard and possibly also in casein
Oxidised vitamin A	Duboulez	Promotes growth	No independent confirmation
Shrimp factor (Astaxanthin ?)	Grangaud	Cures xerophthalmia Less potent in supporting growth	No independent confirmation
Copepod factor	Lane	Substance absorbing at 310 mμ gives rise to vitamin A on incubation with tissues	No independent confirmation
Vitamin A acid	Arens and Van Dorp	Strong vitamin A activity without spectroscopic absorption or chemical reactions of vitamin A	Independently confirmed

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PART VI

THE PATHOLOGY OF VITAMIN A DEFICIENCY OR EXCESS

CHAPTER 24

General Introduction to the Pathology of Vitamin A Deficiency

The pathological effects of vitamin A deficiency are so numerous and diverse that it is difficult to group them together in a clear and unified picture. In early investigations, however, xerophthalmia was the outstanding sign of deficiency, being too obvious to escape notice either in man or in animals. There was therefore an understandable tendency to describe vitamin A as the "antixerophthalmic vitamin" in the same way as vitamin C is described as antiscorbutic and vitamin D as antirachitic. Xerophthalmia is derived from the Greek words "xeros" meaning dry, and "ophthalmos" meaning eye The literal meaning of "antixerophthalmic" is therefore "anti dry eyes".

It was soon realised, however, that lack of vitamin A can cause widespread injuries to the mucous membranes throughout the body. These injuries, moreover, can become the foci of infection by saprophytic bacteria. For a time the description "anti-infective" vitamin gained favour [1] but Harris, Innes and Griffith [2] pointed out that there was no direct antibacterial action The designation "antikeratinising" factor, which implied a power to prevent the hardening of membranes, therefore seemed more appropriate.

More recent research, however, has demonstrated that the keratinisation of membranes is only one aspect of the whole syndrome of vitamin A deficiency With our attention already directed to the eyes we may note, in addition to xerophthalmia, the possibility of at least three other distinctly different forms of injury. Thus absence of vitamin A from the retina will prevent the formation of rhodopsin, and so lead to faulty dark adaptation. Inadequate provision of vitamin A for the growth of the bones and nervous tissues may lead to constriction or twisting of the optic nerve, and so cause complete blindness Finally deficiency of vitamin A in the foetus may lead to microphthalmus, among other congenital abnormalities

Injury to the eye, therefore, can occur in at least four different ways. Since rhodopsin has so far been detected only in the retina its absence constitutes a lesion specific for the eye, but the other three forms of injury may affect various parts of the body. Secondary effects may follow, such

References p 300

as the blockage of ducts with cellular debris the formation of stones or the bacterial invasion of membranes which has already been mentioned. In practice of course each individual cannot be expected to develop into a museum piece showing all the possible lesions There will usually be time before death or successful therapy for the development of only a short selection of lesions The final pathological picture will be influenced by species the duration of deficiency the nature of the diet, the bacterial environment sex and stresses such as variations in temperature. An attempt to summarise and classify the various lesions is made in Table 37

TABLE 37

LESIONS SUSTAINDED IN VITAMIN A DEFICIENCY ARRANGED MAINLY ACCORDING TO THE ORGAN OR FUNCTION AFFECTED THE ANIMALS MENTIONED ARE THOSE IN WHICH THE ABNORMALITY HAS BEEN MOST EXTENSIVELY STUDIED AND THE OCCURRENCE OF THE ABNORMALITY IN OTHER ANIMALS IS NOT PRECLUDED

	Abnormality	*Animal*	*Chapter*
General	Cessation of growth	Rat fowl etc	5 34
	Failure of appetite		
	Decline in body weight	Rat	5 34
	Infections in membranes	Rat	25
	Death		
Eyes	Night blindness	Human farm animals	22 29 31 34
	Xerophthalmia	Human rat	25 31
	Flecking of conjunctiva	Human	31
	Keratomalacia	Human rat	25 31
	Pyogenic infections of eyes	Human rat	25 31
	Opacity of cornea	Human rat	25 31
	Loss of lens	Human rat	25 31
	Papilloedema	Bovine	26 34
	Constriction of optic nerve	Bovine dog	26 34
Respiratory system	Pneumonia	Rat	25
	Lung abscesses caseation	Rat	25
	Metaplasia in nasal passages	Fowl	34
Intestinal tract	Metaplasia in fore stomach	Rat	25
	Achlorhydria (?)	*Human*	25
	Enteritis	Rat	25
Urinary system	Cystitis	Rat	25
	Thickening of bladder wall	Rat	25
	Stone formation	Rat	25
	Pyelitis	Rat fowl	25
	Nephrosis	Rat	26
	Pus in ureters	Rat	27
Liver	Metaplasia in bile ducts	Rat	25
	Calculi in bile ducts	Rat	25
	Degeneration of Kupffer cells	Rat	27

Table 37 (*continued*)

	Abnormality	Animal	Chapter
Skin	Untidy hair or feathers	Rat farm animals birds	25 34
	Hyperkeratosis	Human	31
	Plugging of hair follicles	Human	31
Nervous system	Incoordination	Rat bovine	26 34
	Paresis	Rat pig	26 34
	Nerve degeneration	Rat dog rabbit bovine bird	26
	Constriction at foramina	Bovine dog	26
	Twisting of nerve	Bovine	26
	Hydrocephalus	Rabbit	26
	Abnormal response to stimulation	Rat G pig	26
Bone formation	Defective modelling	Dog	26
	Cancellous bone	Dog	26
	Restriction of brain cavity	Dog	26
	Narrowing of foramina	Bovine	26
	Raised cerebro spinal fluid pressure	Bovine	26
Defective reproduction	Degeneration of testes	Rat	36
	Abnormal oestrous cycle	Rat	36
	Resorption of foetuses	Rat	27
Congenital abnormalities	Anophthalmia	Pig rat	27
	Microphthalmia	Pig rat	27
	Cleft palate	Pig rat	27
	Aortic arch deformities	Rat	27
	Kidney deformities	Rat	27
	Hydrocephalus	Rabbit	27
Miscellaneous	Dental depigmentation	Rat	25
	Cystic pituitary	Bovine	34
	Changed resistance to parasites	Farm animals rat	34
	Arneth right shift (?)	Rat human	27

The depletion of reserves When an animal subsists upon a diet deficient in vitamin A the signs of avitaminosis are postponed until the reserves originally present in the liver are used up With adult animals which have been subsisting upon an adequate diet or in young animals which have had access to a concentrated source of the vitamin even for a short time the reserves may be substantial and the depletion period correspondingly long Thus normal adults in Britain have reserves sufficient to last for at least a year by special feeding rats may be made to accumulate reserves sufficient for about a century Since mammals are usually born with low reserves however they can be made deficient in the vitamin much more readily early in life We shall see that it is even possible for severe injuries to be sustained in the early stages of foetal development

References p 300

Two points of interest deserve attention Firstly it has been found that experimental rats may often continue to grow for several weeks after examination of the livers of their litter mates has shown their reserves to be exhuasted This is one of the arguments suggesting that vitamin A may exist in concealed forms (Chap 23) Secondly it appears that when animals which originally possess high reserves of the vitamin are restricted to a deficient diet they may sometimes show acute signs of deficiency while small amounts of the vitamin can still be detected in the liver Such observations have been made not only on rats [3] but also on cows [4], sheep [5], and turkeys [6] Possibly in prolonged deficiency in older animals the mechanisms for the mobilisation and transport of vitamin A break down before the last traces of vitamin A in the liver are used up

Partial deficiency Ample evidence is available to prove that animals which are kept alive for indefinite periods by small doses of vitamin A may suffer severe effects from partial deficiency In rats life may be supported by doses that leave the teeth in a continual state of depigmentation In males partial deficiency will cause atrophy of the testes, while females will resorb their litters or give birth to deformed young Severe congenital abnormalities may also occur in pigs (see Chap 27)

In young rats and other animals a state of partial deficiency is also indicated as with most other vitamins, by a slow rate of growth

Delayed effects of deficiency In criticising the usual biological method for *estimating vitamin A, in which depleted rats* are given curative doses, Richards and Simpson [7] pointed out that unduly low growth responses may be given by those rats which have already sustained serious injuries It is certainly true that when deficient rats have developed some severe secondary infection, such as pneumonia, they may not only fail to give the appropriate weight response after dosing but may fail to survive at all Even when the injuries which have been sustained are not so acute as to cause an immediate decline, however, they may still have a delayed action on the progress of recovery Thus after dosing growth may be resumed for a time, but later the animal will stop growing and die In the author s experience these temporary recoveries often occur in rats which later are found to have developed stones in the bladder (see Chap 25)

A special case in which the effects of deficiency are delayed in their manifestation after dosing, rather than aggravated by the continuation of the pathological processes, occurs in the continously growing incisor teeth of the rat As explained in Chapter 25 the damage to the teeth which is caused during deprivation of the vitamin only becomes visible above the gums some weeks after growth and health have been restored by adequate dosing

There are grounds for suspecting that blindness may develop in bovines

as the delayed result of abnormalities in the bones of the skull caused by a period of vitamin A deficiency earlier in their history (Chap 34)

Secondary or 'conditioned deficiency

Lesions due to deficiency of vitamin A, as with most other vitamins may sometimes result not from its absence from the diet, but from defects in its absorption or metabolism Thus in an early review on the occurrence of xerophthalmia in Danish children Blegvad[8] distinguished between a majority of cases in which the diet was lacking in the vitamin and a minority in which the diet appeared to be adequate These exceptional cases suffered from digestive abnormalities or other severe diseases It has since been demonstrated by numerous workers that the metabolism of vitamin A may be profoundly affected in different ways in many diseases (see Chaps 31, 32, 34) Thus absorption of the vitamin from the intestines is defective in coeliac disease, sprue and certain forms of liver disease Acute fevers lower the level in the blood and probably eventually reduce the reserves in the liver In some diseases and notably in chronic nephritis and cirrhosis of the liver, the liver reserves may completely disappear Loss of the vitamin in the urine occurs in pneumonia, chronic nephritis and other conditions More evidence is still required as to how often lesions due to vitamin A deficiency occur as secondary effects of common diseases and if they do how much they influence the progress of the disease No doubt can remain, however of the striking effects that disease may exert on the metabolism of the vitamin

Mention may also be made here of the action of vitamin E in protecting the vitamin A reserves of the liver The rapid disappearance of vitamin A from the liver in vitamin E deficiency has been demonstrated in rats (see Chap 20)

Conditions of stress

As already mentioned the amounts of vitamin A which are laid down in the liver seem very large in relation to the minimum daily requirement necessary for growth It is an attractive hypothesis that these reserves, in addition to providing for normal future requirements, may be called upon to meet emergencies in which the vitamin is used up abnormally rapidly, or is required in abnormally large amounts

The stress on the vitamin A status caused by fever has just been mentioned Investigations described in Chapter 38 suggest that increased amounts of vitamin A are required for the efficier[illegible]
There is also some evidence[9] that [illegible]
necessary to maintain life is increas[illegible]

Special aspects of vitamin A deficiency

The purpose of the next three chapters is to give a general account of the effects of avitaminosis A, as seen in various species Emphasis will be placed on

References p 300

experimental studies rather than on clinical or field observations In other parts of the book additional information will be found on vitamin A deficiency in the human subject (Chap 31) and in farm animals (Chap 34) The effects of vitamin A deficiency on sex organs will be discussed in Chap 36

According to the same plan experimental studies on hypervitaminosis A will be described in Chapter 28 while Chapter 33 will deal with the effects of excessive vitamin A and carotene in the human

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CHAPTER 25

Xerophthalmia and Other Epithelial Lesions

The first serious attempt to describe the underlying tissue changes in vitamin A deficiency as indicated to the naked eye by the appearance of xerophthalmia in rats was undertaken in 1922 by Mori[1] Although the internal organs were included in his study he regarded the changes in the lacrimal glands as being the most important feature of the condition Evidence of loss of secretory power was seen in a shrinkage of the cells, and the keratinisation of the cornea and conjunctiva was attributed to the resulting dryness He also noticed similar xerotic changes in the mucous membrane of the larynx and trachea and in the ducts in the salivary glands The Harderian glands, behind the eye ball were atrophied In the opinion of Yudkin and Lambert[2,3], however, the xerotic changes were not a primary effect of deficiency, but were due to a low grade inflammatory process originating in the palpebral conjunctiva, and spreading to the cornea Other workers have reported that in the cow excessive lacrimation, rather than dryness of the eyes can result from deficiency (Chap 34)

Much more detailed studies were described in 1925 in a classical paper by Wolbach and Howe[4] Rats were given a diet consisting of casein, starch salt mixture lard, and brewers' yeast This composition, on modern standards, might be criticised for the absence of vitamins E and D and for the presence of lard, which has recently been claimed to contain some substance having feeble vitamin A activity (Chap 23) There can be no doubt however that the lesions sustained were predominantly due to deprivation of vitamin A The essential effect of this deficiency, as seen in the eyes and paraocular glands, and in parts of the respiratory, alimentary

ibstitution of the normal epithelium by

im the greek "keras" meaning "horn" implies a hardening of the epithelial surface, as in the horny outer layer of, skin The growth of the epithelium is not diminished, but greatly augmented with the formation of a thick layer of squamous cells As they are pressed

References p 312

outwards these cells lose their nuclei and desquamate, or peel off, in the form of scales or sheets Occasionally the numbers of mitotic figures and the reactions seen in the connective tissue and blood vessels might suggest the acquisition of neoplastic properties In view of the changed nature of the surface of the epithelium the description "metaplasia" is often applied.

Xerophthalmia and Associated Eye Lesions

In the author's experience xerophthalmia occurs in most rats given a diet deficient in vitamin A The eye lesions, however, are irregular in their incidence In some experiments all the animals may be severely affected, but in others death from other causes may occur without the development of xerophthalmia Healthy young rats, when awake, have bright widely opened eyes, which appear round and slightly protruding Vitamin A deficiency, in common with other nutritional defects, first causes a narrowing of the space between the eyelids The eyes appear more almond shaped, more deeply set and less bright The next stage, which is typical of vitamin A deficiency but not specific, is the appearance of a blood stained exudate round the edges of the lids By coagulation and drying the exudate soon forms crusts, which often bind the eyelids together, and so make opening of the eye difficult or impossible This condition may sometimes be observed in mild form in rats which have not been deprived of vitamin A, but in advanced avitaminosis the crusts are thick and persistent It is next noticed that the surface of the eye, when it can be seen at periods during which the crusts have broken down, is rough and irregular, with the cornea prominent and dull This appearance is indicative of keratomalacia, which implies ulceration or "softening" of the cornea and conjunctiva Frequently the cornea is lost by sloughing The eye-ball, moreover, may be invaded by pus-forming organisms, which will cause the eye to swell up and protrude outside of its socket The lens may be extruded This stage is presumably a rare occurrence except in deficiency of vitamin A If the rat is dosed with the vitamin most of the early abnormalities may be cured Even the distended eye may regain a more or less normal appearance after the pus has escaped by bursting out, but blindness will seldom be avoided Gudjonsson [6] published an excellent series of photographs taken at different stages of xerophthalmia in rats A selection of these photographs is shown in Plate 7

According to Wolbach and Howe [4] the first histological change in xerophthalmia is keratinisation of the cornea and conjunctival epithelium, which precedes both the cessation of lacrimation, given priority by Mori, and bacterial invasion, given priority by Yudkin and Lambert Later the mucous cells of the conjunctiva become overlaid with keratinised cells, and then













Plate 7 Stages in the development of xerophthalmia in the rat (Gudjonsson) (1) Normal eye (2) Early symptoms in a rat which has been deprived of vitamin A for 28 days Shrinkage of the Harderian gland behind the eye (not seen) has caused the eye ball to sink more deeply into its socket (Enophthalmus) A little blood stained exudate has appeared round the eye (3) On the 31st day of deficiency the amount of exudate has increased (4) On the 55th day the eye is almost completely closed (5) On the 69th day the eyelids are firmly stuck together by dried exudate On this day the rat died The autopsy revealed abscesses at the base of the tongue cystitis and pyelonephritis (see page 307) (6) The eye in another rat which has been cured of xerophthalmia by dosing with vitamin A Note the opacity of the cornea which caused blindness This complication is comparatively rare

atrophy and disappear Accumulations of detached keratinised cells from the conjunctival sac adhere to the margins of the eyelids, and the basal cells on the conjunctival epithelium show slight irregular downgrowth Increased vascularisation of the underlying connective tissues is indicated

References p 312

by the appearance of additional capillaries, presumably as a physiological response to the increased demands of the rapidly growing abnormal epithelium which has replaced the normal corneal epithelium Accumulations of mononuclear cells, lymphocytes and endothelial leucocytes are common at this stage Even if there is no definite evidence of infection polymorphonuclear leucocytes invade the keratinising epithelium The destruction of the cornea by infection, however, is regarded as accidental, and need not occur even when the deficiency has been "carried to an end result"

Epithelial Lesions in Other Sites

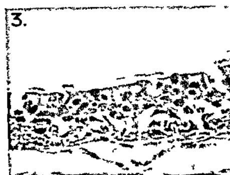
The respiratory tract In the trachea an early sign of vitamin A deficiency, again according to Wolbach and Howe [4], is seen in the appearance of clumps of two or more deeply staining cells below the surface of the original epithelium, which is mainly columnar Later these cells form a ring completely circling the lumen, although the surface of the epithelium still remains normal Eventually the original epithelium may be cast off in sheets, and replaced by keratinised cells The nares and bronchi also become keratinised Pneumonia often develops, and is one of the common causes of death When the deficiency has run an acute course the lungs usually appear dark red through congestion with blood A different picture, however, is often seen in animals which have survived a long period of deficiency or partial deficiency Invasion by pyogenic bacteria is indicated by the presence of pockets of pus with widespread necrosis and the caseation of whole lobes, or considerable parts, by the accumulation of cellular debris

A series of photomicrographs showing different stages of abnormality caused by vitamin A deficiency in the trachea of the rat is shown in Plate 8 The sections were kindly supplied by Dr D L Wilhelm who has also studied the regeneration of the tracheal epithelium after curettage [6]

Intestinal tract In their investigation Wolbach and Howe [4] noticed only a slight degree of atrophy in the oesophagus, stomach and intestines of a few of their animals Later they reported, however that the fore stomach which is normally covered with a squamous epithelium, often shows thickened areas of hyperkeratosis [7] This finding was in agreement with earlier work by Fujimaki [8] and was subsequently confirmed and extended by other investigators [9–13] The changes tend to occur near the boundary of the squamous and glandular parts of the stomach and apparently commence as areas of atrophy These regions become surrounded with rings of hypertrophy, possibly through infection, and ulcers or papillomata may eventually be formed According to Fujimaki, however, these lesions could be intensified by increasing the fat content of the diet, and by great excess of fat could be produced even in animals given cod liver oil











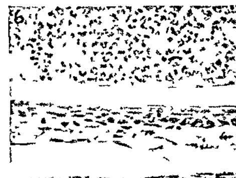


Plate 8 Changes of increasing severity in the tracheal epithelium of rats deficient in vitamin A All the photomicrographs have been taken at the same magnification, and with the lumen uppermost (1) Normal epithelium Note the regular, columnar arrangement of the cells all of which have cilia pointing into the lumen (2) Very early *changes after 3 weeks of deficiency Note the crowding, and derangement of the* epithelial cells The cilia are missing in several places (3) More severe changes of the same character (4) After 4 weeks of deficiency a raised area of metaplasia is seen, which is made up of superimposed stratified cells (5) Squamous metaplasia After 5 weeks of deficiency the original columnar epithelium has been completely replaced *by flattened, stratified cells Sheets of keratin are peeling off into the lumen,* where there is evidence of bacterial infection (6) A later stage, in which the whole lumen is blocked by cellular debris and the products of infection

References p 312

It would appear therefore that the vitamin A deficiency is not the only dietary defect which can provoke this form of injury Gastric ulceration has indeed been reported *inter alia* in deficiencies of vitamins B_1 [14] and C [15]

Richards [13] claimed that intestinal abnormalities could be seen by the naked eye in rats which had been deprived of vitamin A for only three weeks In addition to injuries to the squamous part of the stomach she noticed pittings points of haemorrhage and sometimes ulceration in the glandular part These injuries might be expected to effect the secretion of mucus and acid and therefore give interest to claims that in the human vitamin A may sometimes correct achlorhydria [16–18]

Abnormalities in the lower ileum were described by Cramer [19] in 1923 The villi were atrophied and necrosed at their tips A further examination [20] revealed that the intestinal mucous glands were atrophied to an extent which must have interfered seriously with their activity Deficiency in mucus led to the uncontrolled multiplication of bacteria which extended beyond their normal habitat in the lumen to penetrate between the villi and infest the crypts of Lieberkuhn and the caecal mucous glands Later De Ruyter [21] confirmed Cramer s observations and also reported the disappearance of goblet cells from the intestines

Diarrhoea and sometimes colitis and ulceration of the colon have been reported as effects of vitamin A deficiency in many animals [13 22 7] Recently Mayer and Krehl [23] commented on the frequency of a bloated intestinal tract as a *post mortem* finding in rats which had died from vitamin A deficiency, especially if vitamin C had been supplied This condition was attributed to the blocking of the oesophagus with cellular debris The author s experience confirms that intestinal abnormalities including both distension with gas and the appearance of blood in the intestinal contents are a frequent cause of death in deficient rats Often the animal may succumb before any of the more characteristic lesions such as xerophthalmia and infections with pyogenic bacteria have time to develop

Salivary glands Wolbach and Howe [4] studied the submaxillary glands the accessory salivary glands at the base of the tongue and pharynx and the parotid glands of deficient rats The first change to be noticed was a slight atrophy of the acini both in mucous and serous types with atrophy of the epithelium of the duct Islands of keratinising cells appeared before there was any evidence of infection and in the capsule there was a temporary stage of oedema As avitaminosis progressed the keratinisation of the ducts and atrophy of the gland tissue was intensified usually with the complication of infection Swelling of the sublingual glands with pus is indeed sufficiently common to be counted among the more familiar and characteristic effects of experimental vitamin A deficiency

In the bovine Jungherr Helmboldt and Eaton[29] have reported that the parotid gland is especially prone to exhibit specific histo pathologic alterations in hypovitaminosis A They consider that it is the only organ so far ascertained which lends itself to the specific morphological diagnosis of vitamin A deficiency in this animal

The urinary tract Wolbach and Howe[4] found that in their deficient rats the normal epithelia of the bladder the ureters and the pelvis of the kidney were replaced by keratinised epithelia which developed from underlying nests of cells as already described for the trachea The change in the nature of the epithelium was accompanied by remarkably rapid growth with the appearance of numerous mitotic figures In the ureters pelves and bladder there was often a downgrowth of epithelium with the incorporation of blood vessels in the bladder these processes produced invaginations and formations like dermoid cysts These abnormalities were considered to be the most remarkable observed throughout the investigation and to be suggestive of neoplastic potentiality

Similar observations have since been made by many other workers Tyson and Smith[30] commented on the early appearance of the changes The first stage appeared to be a piling up of the epithelium with infiltration of the subepithelial tissues with neutrophiles as a second stage the epithelium became keratinised Harris Innes and Griffith[31] noticed hyperplasia in the kidney pelvis In the bladder Arons and Van der Rijst[11] found that the metaplastic changes started at the urethral orifice and extended to cover the whole epithelium of the bladder in animals subjected to prolonged deficiency

The main complications of vitamin A deficiency in the urinary system as seen in the rat are prevention of micturition through blockage of the urethra nephrosis infection of the ureters and kidneys with pyogenic organisms and stone formation These secondary effects of course are often superimposed on each other but the pathological picture may range from an acute and rapidly fatal production of pus throughout the urinary system to the slow formation of stones in the course of prolonged partial deficiency

Frontali[32] Richards[13] and Bliss Livermore and Prather[33] may be mentioned for their early work on urinary infections in vitamin A deficiency In the author s experience whole groups of rats exposed to vitamin A deficiency may die from pyelitis and cystitis after which other groups may be examined in which urinary infections are much less in evidence Such variations as already suggested may be due to changes in the bacterial environment In typical cases the pelves of the kidneys may be filled with pus and abscesses may be formed in the kidney tissues The ureters become enormously

References p 312

distended with pus, and the bladder filled with mixed pus and urine In other animals stoppage of urination may be more prominent than pus formation Debris from the greatly thickened bladder wall may block the urethra, with the result that the bladder is distended with blood stained urine until it reaches almost to the diaphragm

According to Fujimaki[8] and Higgins[34] infection causes the urine to become alkaline The effects of vitamin A deficiency on the urinary tract in birds are reviewed elsewhere (Chap 34)

Urolithiasis As early as 1917, before the differentiation of vitamins A and D, Osborne and Mendel[35] suggested that calculosis was related to deficiency of the fat soluble vitamin In autopsies on 857 rats calculi were found in 81, all of which had a history of vitamin A deficiency Fujimaki[36] confirmed that calculi could readily be produced by giving a diet deficient in the vitamin Van Leersum[37] followed with histological and radiological studies Calculi were absent from 241 normally fed rats Out of 297 male and 348 female vitamin A deficient rats, however, calculi were found, either by naked eye or by the microscope, in 130 males and 67 females

Van Leersum commented that the greater incidence of calculi in males than in females must be due to their longer urethra but the well established differences between sexes in the metabolism of vitamin A (Chap 35) may perhaps suggest that other factors may also be involved He found that the urine was usually acid There was no obvious infection but haematuria was frequent The deposits appeared to originate in the calcification of keratinised epithelium in the kidney tubules Casts were then dislodged and served as foci for calcification in the kidney pelves, ureters and bladder The earliest calculi appeared after 17–19 days of deficiency, and they were common after 21 days A stone in the kidney measuring 2 mm by 1 mm could be detected radiologically and was considered to be large Most of the calculi consisted of calcium phosphate or ammonium magnesium phosphate, but concrements of calcium oxalate were also common

In the author s experience young rats which are given a diet deficient in vitamin A from weaning usually die before the formation of stones has reached a stage which can readily be detected by the naked eye Stones are often to be found however, in rats which have been exposed to deficiency and subsequently dosed with the vitamin The effect of a small supplement is often to cause a temporary increase in weight, followed by a decline, and on autopsy the greatly thickened bladder is found to hold either one or more large stones or a mass of small "gravel ' Occasionally lithiasis may be seen after heavy dosing, and the urinary lesion may be concomitant with a high reserve of vitamin A in the liver It would appear that once a focus



Plate 9 Distension of the bladder by blockage of the urethra in a rat with a history of vitamin A deficiency The animal was first deprived of vitamin A completely for 2 months and was then given small doses of vitamin A for another 3 months Two small stones about 1 mm in diameter were found In another animal with a similar history the bladder contained at least 40 stones with diameters from 0 5 mm up to about 2 mm (Moore)

for stone formation has been started the process may sometimes proceed even after its original cause has been remedied (Plate 9)

The question of the specificity of the relationship between vitamin A deficiency and urolithiasis has given rise to controversy Bliss [33] eliminated one complicating factor by showing that stones are formed in vitamin A deficiency even when vitamin D is supplied Van Leersum observed stones only rarely in rats given a rachitogenic diet McCarrison and Raganathan [38] however found stones when excess of calcium was given in conjunction with a diet low in protein but adequate in vitamin A McCollum and Simmonds [39] confirmed the effect on stone formation of a high Ca P ratio and Watchorn [40] obtained the same effect on giving excess of magnesium

References p 312

carbonate It seems obvious that urolithiasis may arise from different defects The possibility of an association between stone formation and defective dark adaptation in humans will be mentioned in Chap 31

Genital system In both the testes and the uterus the epithelium is highly vulnerable to vitamin A deficiency Keratinisation of the vaginal epithelium without significant abnormalities in the ovaries appears so early and regularly in animals deprived of vitamin A that it has been used as the basis for biological tests Degeneration of the testes also regularly occurs but is more difficult to observe without killing the animal A detailed account of the lesions in both these organs is given elsewhere (Chap 36) It may be added here that the secondary sexual organs of the rat may occasionally be foci for pyogenic infections This especially applies in the male to the seminal vesicles and the preputial glands and in the female to the *bulbi vestibuli* which correspond to the preputial glands

Liver Particular interest in the effects of vitamin A deficiency on the liver may arise from its importance as the main storage depot of the vitamin It might be argued that the presence of la ge amounts of the vitamin at least during the early stages of its withdrawal from the diet would ensure that the liver would be protected longer than other organs from the effects of deficiency On the other hand the high concentration of vitamin A which is usually present in the liver might suggest that the requirement of the hepatic tissues for the vitamin exceeds those of other tissues

Early investigations gave no indication that the liver is seriously affected by deprivation of vitamin A [41–45] Wolbach and Howe [41] however reported a reduction in size which they attributed to the loss of glycogen and fat Gross [44] noticed congestion Thatcher and Sure [45] observed that in about one third of their deficient rats areas around the portal spaces had become fibrosed De Ruyter [21] and other workers also described abnormalities including injuries to the reticulo endothelial system which will be discussed later (Chap 27)

Hints that the epithelium of the bile ducts may be injured by lack of vitamin A has been given by reports of the formation of stones in the bile ducts or gall bladders of deficient animals Fujimaki [36] described the formation of cholesterol calculi in the bile ducts of rats deprived of the vitamin but not dosed with cholesterol Emiliani and Bazzocchi [46] frequently found gall stones in deficient guinea pigs Much more detailed information however has recently been supplied by Hamre [47] Out of 22 rats which were deprived of vitamin A long enough to exhibit signs of deficiency no less than 19 were found to have dilated bile ducts accompanied by some degree of jaundice Metaplasia of the epithelial lining was observed in all

dilated extrahepatic ducts and in many intrahepatic ducts The ducts became obstructed and calculi of varying sizes and composition were formed but always on a nucleus of detached epithelial cells The obstruction of the bile ducts resulted in a mild hypertrophy of the tissues of the portal tracts and moderate degenerative changes of the peripheral parenchymal tissues of the hepatic lobules

From the above observations it appears that the epithelial cells of the liver are not immune to the general influence of vitamin A deficiency in causing metaplasia and keratinisation It remains to be seen, however, whether the liver is always affected in vitamin A deficiency, or is damaged only under the particular experimental conditions chosen by Hamre

Skin Our evidence of the effects of vitamin A deficiency on the skin relates mostly to humans and is reviewed in detail elsewhere (Chap 31) The skin becomes thick, dry and scaly In some parts of the body the hair follicles are enlarged and blocked with horny plugs

In rats the hair loses its smoothness and the feet become scaly and rough [27], but changes which are at least superficially similar are common in many forms of malnutrition In histological studies Studer and Frey [48] found that vitamin A deficiency caused rats' skin to become atrophic, and abnormally thin

Teeth Most studies of the effect of vitamin A deficiency on the teeth have been made on the rat in which the incisors grow continuously and are kept in their correct shape by grinding down The injuries sustained are not confined to the epithelial cells but affect also the pulp and the odontoblasts Thus in the early stage of deficiency Wolbach and Howe [4] observed shrinkage of the odontoblastic layer with the irregular formation of dentin, while later the odontoblasts completely disappeared In animals which have a long history of survival on a deficient diet, however, the most obvious change is of epithelial origin and can be readily seen in the absence of the deep brown iron containing pigment which usually covers the labial surface of the incisors This abnormality indicates that some weeks previously the enamel forming cells have been in an atrophic state [49–53]

Even the injuries to the odontoblasts, according to Schour, Hoffman and Smith [51] must be considered as secondary effects of epithelial lesions Thus the rat's incisor develops primarily from an elliptical epithelial base which is situated at the proximal end of the tooth, and which proliferates throughout the life of the animal Because of its function it may be described as the odontogenic epithelium The activity of the cells in this base not only regulates the development of the outer enamel-forming cells, but also causes the differentiation of the underlying mesenchymal cells into the odontoblasts Schour and his colleagues also noticed that disorganisation in the functioning

References p 312

of the odontoblasts caused the deposition of an unduly thick layer of dentin on the labial aspect of the tooth

In studying the degree of specificity of dental depigmentation as a sign of vitamin A deficiency Moore [54] found the same bleaching could also be induced by deficiency of vitamin E and called attention to earlier observations of the same abnormality *in fluorosis* [55] *and magnesium deficiency* [56] The common final effect of the disappearance of the brown colour however does not necessarily imply that the underlying histological abnormalities are identical and Irving [57] has distinguished between the injuries in deficiencies of vitamins A and E

In the author s experience the incisors of albino and piebald rats are about equally vulnerable to loss of colour in deficiency of vitamin A In contrast avitaminosis E has been found to cause depigmentation much more readily in albinos than in piebalds [58] [59] In the albino depigmentation is induced less readily by vitamin A deficiency than by vitamin E deficiency

As a further complication it is clear as already hinted that the condition of the surfaces of the teeth which are visible during life do not reflect the current status of the animal in regard to vitamin A but the status as it was several weeks previously Time must elapse before injuries sustained beneath the gums can be brought into view by the growth of the tooth Thus young rats when taken at weaning will be seen to have white incisors since the brown layer does not develop during about the first two months of life After a diet deficient in vitamin A has been given for 5 or 6 weeks, however the teeth will have become brown although at the same time signs of avitaminosis A may be seen in cessation of growth and the appearance of xerophthalmia The external parts of the teeth presumably now reflect the period when the rats still possessed reserves of the vitamin When next the animals have been cured by dosing with vitamin A and rapid growth has been resumed the teeth within a few weeks may become completely white Inspection of only the exposed surfaces of the teeth therefore proves quite deceptive as a guide to the condition of the rat at the time of observation [60]

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CHAPTER 26

Nerve and Bone Lesions

In the experimental rat, which was the subject of many of the investiga
described in the preceding chapter, xerophthalmia and other epit
injuries are the most readily observed effects of vitamin A defici
Xerophthalmia is also one of the most characteristic lesions in the hu
In other species, however injuries to the nerves and bones may accom
the epithelial lesions, and even predominate over them in importance
the bovine sometimes develops xerophthalmia, but injuries to the n
and bones may occur in the absence of this abnormality In pigs the
characteristic lesion is paralysis of the hind legs, usually without xer
thalmia

Nerve Degeneration

Early observations of nerve degeneration

In 1916 Hart, Miller and McCollum [1] observed
of nerve degeneration in pigs which were fed
mixtures of wheat and other grain At that time
theory of deficiency diseases had not been fully developed, and it
suggested that the injuries were due to some toxic principle in the wh
Since alfalfa prevented the lesions it was assumed that the fat-soluble A
minerals which it contained in some way counteracted the toxic actio
the wheat

Twelve years later Hughes, Lienhardt and Aubel [2], who had by then
advantage of a clear knowledge of the difference between vitamins A anc
made a direct approach to the problem of vitamin A deficiency in the
The animals were given at weaning a diet of white maize and tankage,
were allowed access to a sunlit yard to prevent rickets Throughout
investigation the eyes were carefully watched, in the expectation t
xerophthalmia would develop, but in most cases the only abnormality
a slight watering The tissues round the eyes were never involved, and o
one small corneal ulcer was seen in 27 animals After 6–10 months, howev
the pigs all developed a marked nervous disorder, characterised by blindn

incoordination and spasms This condition ended fatally in those animals which were not killed for examination or cured by giving adequate supplies of recognised sources of vitamin A such as cod liver oil butter fat yellow maize and alfalfa meal

Histological studies revealed degeneration of the nerve bundles in the optic thalamus in the optic femoral and sciatic nerves and in certain parts of the spinal cord Evidence of nervous lesions was also found in other species Stiffness and incoordination were noticed in cows which had reached maturity before restriction to a deficient diet In a large group of chicks which were given a deficient diet from hatching 90% developed symptons of severe nerve degeneration

Shortly before Hughes and his colleagues had reported their observations the first of an important series of communications by Mellanby [3] had been given in preliminary form Lesions of the spinal cord were detected in puppies which had received a diet low in vitamin A and containing wheat germ In a detailed report which followed in 1931 [4] it was stated that rye germ was more injurious than wheat germ as a contributory cause of the degeneration and that ergot was more injurious than either The inclusion of sources of vitamin A in the diet prevented or diminished the injuries

The relationship between epithelial and nerve lesions

Mellanby was next attracted by the idea that a close association might exist between the epithelial lesions as indicated by xerophthalmia and the nerve injuries as indicated by incoordination He therefore compared the severity of these two types of injuries as seen in rabbits given a diet of oats wheat bran chalk and alfalfa heated in air to destroy its carotene In animals with xerophthalmia degenerative changes were found in the myelin sheaths of the trigeminal nerve In early and slight xerophthalmia the changes in the nerve were annular and were consistent with the ability of the nerves to recuperate on the restoration of the vitamin to the diet In severe xerophthalmia the nerves showed typical Wallerian degeneration with the invasion of the axis cylinder with degenerated myelin Such lesions were consistent with the nerves being curable only with difficulty In rats in which the eyelids became puffy without severe injury to the cornea it was also found that the trigeminal nerves were degenerated Mellanby suggested that the xerophthalmia might be secondary to a loss of the neurotrophic control normally exerted on the cornea by the ophthalmic division of the trigeminal nerve [5] (Plate 10)

Extensive studies on rats rabbits and fowls by Rao [6] however raised doubts as to this conclusion Rao agreed that xerophthalmia and myelin degeneration of the afferent nerves of the eye could usually be seen together His finding that sometimes the nerve lesions were present without injuries

References p 328

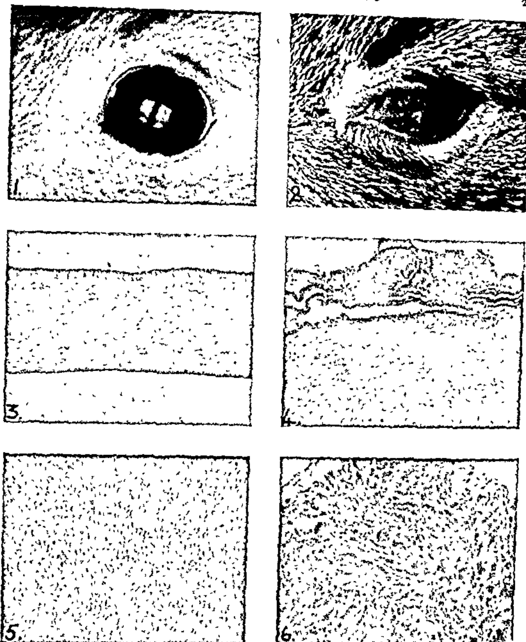


Plate 10 Xerophthalmia accompanied by nerve degeneration in vitamin A deficiency in the rabbit (Mellanby, 1934) Left photographs, Nos 1, 3 and 5 show the eye of a normal rabbit, clearly reflecting a window, with sections of the cornea and trigeminal nerve (1st branch) below. Right photographs, Nos 2, 4 and 6 show the same subjects, but for a rabbit which had been deprived of vitamin A for four weeks Note that xerophthalmia (2) and keratinisation and infection of the cornea (4) are associated with extensive degeneration of the nerve (6)

in the cornea and conjunctiva, moreover, was consistent with the eye lesions being secondary to the nerve injuries. In animals suffering from xerophthalmia, however, no relationship could be found between the severity of the lesions in the eye and in the nerve. In animals in which one eye was

more severely affected with xerophthalmia than the other the injuries in the corresponding nerves showed little difference Complete recovery of the nerve lesions did not occur in animals which were successfully cured of their xerophthalmia by dosing with vitamin A Rao therefore disputed the contention that xerophthalmia is a secondary effect of nerve degeneration

Nerve lesions in animals not given cereals

Rao s experiments on rats were of special interest because the diet had been of the conventional synthetic type with casein starch olive oil dried yeast and minerals as its constituents While there was no reason to discredit the power of coarse cereals in aggravating nervous injuries it was clear that lesions could be incurred even when they were absent from the diet

This conclusion was in agreement with an interesting report on incoordination in vitamin A deficient rats which had been published two years previously by Aberle [7] Weaning rats were given a diet similar to that used by Rao and the times taken for the appearance of the various abnormalities caused by vitamin A deficiency were recorded When the mothers had been given a diet rather low in vitamin A the young rats developed vaginal cornification after 4 weeks of restriction xerophthalmia after 5–6 weeks urinary incontinence after 7 weeks and various graded stages of paralysis after 6–8 weeks with death usually intervening between 8 and 9 weeks The first stage of paralysis was indicated by the rats having their hind legs extended rather than flexed when they were held up by the neck In the second stage there was incoordination of movement and the feet slipped during walking Stage 3 was reached when the feet were placed at a wide angle to the body during both standing and walking and there was great weakness of the hind legs Death usually prevented the development of stage 4 in which the hind legs were dragged helplessly behind when the animal pulled itself forward with its front legs

In young rats which had been bred from mothers given a diet containing vitamin A in the form of table scraps the appearance of all the abnormalities was delayed The nervous symptoms could be prevented by cod liver oil which was usually also effective in curing or improving the lesions once they had developed In histological studies on the animals which had not been dosed with vitamin A degenerative changes were found in the medullary sheaths of the sensory tracts at the periphery In a few instances degeneration had occurred in the posterior nerve routes

Later Zimmerman and Cowgill [8] carried out an essentially similar investigation but with linoleic acid added to their basal dietary to ensure that there should be no deficiency of essential fatty acids They observed the same symptoms of paralysis as had been reported but in less than half their animals Histological studies however usually indicated nerve degeneration

References p 328

even in rats which had not been paralysed Animals which were adequately dosed with carotene prophylactically exhibited neither paralysis nor histological evidence of nerve degeneration, but attempts at curing the nervous lesions were less successful than those of Aberle Often the neurological symptoms persisted even after dosing with carotene had caused the resumption of growth and the curing of xerophthalmia Histological studies on such animals showed that the degeneration of the nerves was more severe than in animals which had not been dosed Presumably resumption of growth placed an additional strain on the damaged nerves, which in any case respond to treatment only with great difficulty

Associated Bone and Nerve Abnormalities

The relationship between bone and nerve lesions

In 1935, soon after the appearance of Aberle's paper, an important communication was published by Lane Moore, Huffman and Duncan [9] on blindness in calves For the previous 12 years they had been investigating the effect of the quality of hay on the health of dairy cows and calves and during this period had observed 24 cases of incurable blindness In affected animals there was no evidence of xerophthalmia, but the pupils remained dilated in intense sunlight and the blindness was often accompanied by a partial paralysis, particularly of the front quarters, weakness spasms and a peculiar position of the head The cases of blindness were noticed either (1) in calves which were given diets containing poor quality roughage from an age of about 3 months onwards or (2) in the recently born offspring of cows given similar diets In the older calves the blindness was usually noticed after the defective diet had been given for 6–9 months Calves from mothers which had been given the defective diet were sometimes blind even at birth

Moore and his colleagues reviewed the considerable literature which was already available on blindness in bovines, and concluded that it could be incurred in three distinct ways Firstly it could result from xerophthalmia followed by xerosis and infection of the cornea, according to the familiar picture in the human and the experimental rat Secondly it could be caused by papilloedema or choked disk, in the retina Thirdly it had been shown to result from degeneration of the optic nerve [10] The cases which they now described were shown to be due to the third cause and it was demonstrated that the precise point of the injury was at the optic foramen where the nerve was atrophied by pressure of the bone which did not allow a sufficiently wide aperture for the passage of the nerve (Plates 11 and 12)

The relationship of the nerve injuries to the development of bone led Moore to enquire whether rachitic changes might be involved, but blindness

occurred in calves exposed to sunlight or dosed with vitamin D Estimations of calcium in the blood moreover gave normal results On the other hand the basal rations of the affected animals were always low in vitamin A

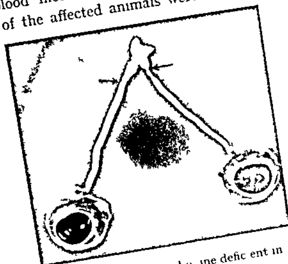


Plate 11 Constriction of the optic nerve in a bovine deficient in vitamin A Photograph dissected eyes and nerves (Lane Moore)

The administration of supplements of the vitamin from an early stage gave protection although blindness sometimes occurred in animals which had been given supplements after a preliminary period of deficiency

On the evidence then at his disposal Moore seemed reluctant to believe that vitamin A deficiency could be responsible for abnormalities in bone,

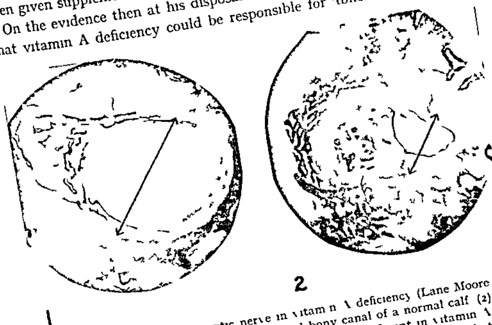


Plate 12 Constriction of the optic nerve in vitamin A deficiency (Lane Moore *et al* 1935) (1) Cross section of the optic nerve and bony canal of a normal calf (2) Optic nerve and canal of the blind new born calf from a cow deficient in vitamin A Same magnification Note the smaller diameter of the canal as compared with that of the normal animal

References p 378

and for the time remained undecided as to the nature of the dietary defect which was responsible for the blindness He mentioned the possibility, however, that deficiency of vitamin A might raise the intracranial pressure, and so narrow the optic foramen as a secondary effect

Degeneration of the auditory nerves

From blindness in calves the front line of progress turned next to deafness in dogs In following up his theory that the primary lesions in vitamin A deficiency were to be found in the nerves Mellanby [11] continued his studies on dogs, and in 1937 reported that destructive changes in the ganglion cells of both branches of the 8th nerve often caused complete deafness A year later [12] however, he noticed that in addition to the nerve degeneration there was overgrowth of the bone in the labyrinthine capsule Bone in the modiolus of the cochlea had hypertrophied, and so caused pressure on the fibres of the 8th nerve There was also overgrowth of the periosteal bone of the capsule at the exit of the internal auditory meatus This had the effect of lengthening the distance from the cochlea to the brain and so stretching the nerve

Examination of the labyrinthine capsule and its nerves suggested that the overgrowth of bone at least in this anatomical region, was responsible for the nerve degeneration In his previous work, however, Mellanby had noticed widespread degeneration in the afferent nerves of the body both cranial and somatic He therefore looked for further evidence of the influence of faulty bone formation in other sites, and found that the injuries to the optic and trigeminal nerves could be ascribed to the overgrowth and deformity of bone at the base of the skull

Mellanby, who appears to have been unaware of the American work on calves at this time, was confident that vitamin A was the main dietary defect involved He persisted in the view, however, that the injuries were aggravated by the presence of cereals in the diet In agreement with this conclusion he found that when potatoes were substituted for cereals in the diet of his puppies the severity of their injuries was greaty reduced

Increased intra cranial pressure

Soon after the appearance of Mellanby s communications Moore [13] completed careful studies which enabled him to agree that the bone lesions were caused by vitamin A deficiency He confirmed that when calves were given a diet low in carotene they developed night blindness followed by papillary oedema and permanent blindness caused by constriction of the optic nerve All these abnormalities could be prevented moreover, by the administration of crystalline carotene in oily solution In view of the consistent occurrence of syncope spasms and incoordination in the deficient animals he renewed his suggestion that the intracranial pressure must be increased

The matter was soon put to test by Moore and Sykes [14] who observed increased spinal fluid pressures in calves suffering from moderately severe deficiency of vitamin A A further investigation followed in which calves were made to suffer prolonged periods of partial or complete deficiency for the special purpose of finding how high the pressure would rise The measurements were made by making a puncture without anaesthesia into the subarachnoid space Pressures rose from a normal level of about 100 mm of saline to 400-600 mm and were influenced to some extent by excitement The severity of papilloedema and incoordination and the liability to syncope and convulsive seizures went parallel to the cerebro spinal fluid pressure When the intracranial pressure was raised by exciting the animals con vulsions or fainting were often precipitated

Later Moore and his colleagues [15] investigated the vitamin A requirements of calves by giving the animals graded allowances of carotene for prolonged periods and measuring the pressure in the cerebro spinal fluid Doses of dried alfalfa meal equivalent to 30–32 μg of carotene per lb of body weight daily allowed the pressure to remain normal but with doses of 28 30 μg abnormally high pressures were observed The degree of accuracy claimed for the method was surprising and it would be interesting to know whether the same accuracy can be maintained in repeated experiments on larger groups of animals

The regulation of bone modelling by vitamin A Moore s first measurements of the intracranial fluid pressure in calves were soon followed by similar measurements by Mellanby in puppies [16] He found that in deficient animals it was sometimes difficult to make a clean puncture into the cisterna magna which often contained a reduced quantity of fluid In four dogs which were successfully examined however an average pressure of 100 mm of water was found as compared with 58 mm in animals which had been dosed with vitamin A Mellanby also commenced detailed studies of the histological changes underlying faulty bone formation which occu pied his attention for several years [17] The cause of the abnormalities was traced to increased and disorganised activity of the osteoblasts and osteo clasts As a result the modelling of the bones was disturbed with a tendency for the formation of cancellous rather than compact bone Thus in place of normal hard bones larger soft bones were formed Chemical analyses however indicated little difference between the total calcium contents of the normal and the diseased bones

Although deficiency caused disorganisation in the formation of bone Mellanby emphasised that the activities of the osteoblasts and osteoclasts were not anarchic but orderly in so far as they conformed to abnormal patterns Thus their activity at effective surfaces was modified or even

References p 328

reversed with the deposition of bone in places which would normally have shown no activity or from which bone would have been absorbed While the details of the pathological processes varied in different regions the end effect was always a thickening and dysplasia of the bone (Plates 13 and 14)

When vitamin A was restored to the diet of the puppies while they were still growing the abnormalities in bone formation were corrected Details of the processes of curing as for the development of the lesions varied in different anatomical regions When the altered shape of the bones had caused injury to the adjacent nerves the recovery of the bones was not affected by the condition of the nerves

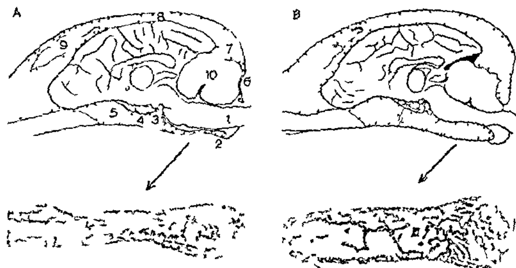


Plate 13 *Above* Drawings by Mellanby (1944) of mesial sagittal sections of skulls of litter mate dogs of the same age (A) adequate in vitamin A (B) deficient in vitamin A Note the thickening of the bones in the deficient animal particularly surrounding the posterior fossa The brain is compressed and the cerebellum is pushed back into the foramen magnum (1) Foramen magnum (2) Basi occipital bone (3) Posterior clinoid process (4) Basi sphenoid process (6) Supra occipital bone (7) Occiput (8) Parietal bone (9) Frontal bone (10) Posterior fossa
Below Semi diagrammatic drawings of the basi occipital bones The osteoclasts are represented by black spots It will be seen that the bone is thickened in deficiency In the normal bone a large number of osteoclasts are located on the surface of the bone which is adjacent to the brain In the abnormal bone osteoclasts are absent from the external surface of the bone but are abundant on the underlying marrow surface The failure of the osteoclasts to allow for growth by removing the upper layers of the bone and their abnormal activities inside the bone result in faulty modelling and a loose cancellous structure

The unbalanced growth of nervous tissues

In spite of the comprehensive character of Mellanby s careful work an alternative theory to explain the relationship between bone and nerve injuries deserves mention By taking young rats from mothers which had

been deprived of vitamin A during the later stages of lactation and by submitting them after weaning to the same deficient diet for 6–9 weeks Wolbach and Bessey [18] produced the signs of incoordination which had been

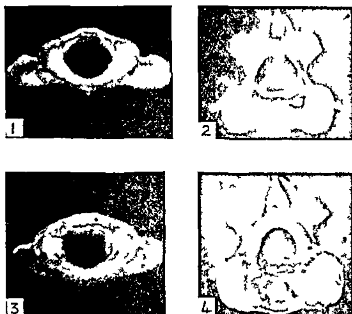


Plate 14 Atlas and dorsal vertebrae from dogs (1 and 2) adequate and (3 and 4) deficient in vitamin A Note the narrowing of the foramen in the deficient dog (Mellanby 1944)

reported earlier by Aberle Histological examination revealed that the lesions were of mechanical origin and unrelated to the epithelial changes Overcrowding of the cranial cavity had caused distortion of the brain and dislocation towards the foramen magnum with herniation of the cerebellum in the foramen There was also multiple herniation of the cerebrum and cerebellum in the venous sinuses of the dura Overcrowding of the spinal canal similarly caused distortion of the cord and herniations of the nerve roots into the vertebral foramens and into the body of the vertebrae

As a simple explanation of these lesions it was inferred that vitamin A deficiency arrested the growth of bone but that the nervous tissues continued to grow at a normal rate Mellanby pointed out however that the prime factor in growth is the laying down of bony tissues by osteoblasts This process is not stopped by deficiency of vitamin A although its place of action may be altered Wolbach and Bessey observed no compression of the nerves in rats whose growth had been stunted by partial starvation or by deficiencies of riboflavin or of vitamin B_6

Studies of the neurological manifestations in ducks deficient in vitamin A at first led Fletcher and Rigdon [19] to support the view that damage to the

References p 328

nerves is caused by compression exerted by the bones A later investigation by Rigdon [20] however indicated that the primary lesion causing ataxia was a degeneration of the cells of the cord particularly in the anterior horn which could not be related to abnormalities in the bone Adamstone [21] distinguished between chicks deprived of vitamins A and E In vitamin A deficiency there were no gross lesions but pin point areas of degeneration were found in the brain stem the base of the cerebellum the optic chiasma and *occasionally in the cerebrum* In contrast vitamin E deficiency caused general disintegration of the cerebellum with yellow discoloration haemorrhage and oedema microscopic examination showed widespread degeneration of the neurons

Yet another conclusion as to the exact cause of the nervous degeneration in vitamin A deficiency was reached as the result of research on bovines by Blakemore Ottaway Sellers Eden and Moore [22] In their experience there was no direct pressure on the optic nerve by the bone As the result of a disorganised balance of growth between nerve and bone the optic nerve became unduly long which caused it to twist and kink Abnormal proliferation of the sheath at the point of kinking then led to a complete break in the nerve with a capping of the divided ends which were only joined by loose connective tissue (Plate 15)



Plate 15 Degeneration of the optic nerve in a bullock deficient in vitamin A (Blakemore *et al* 1950) The nerve was completely divided Each broken end was capped by proliferation of the nerve sheath The ends were joined together only by loose connective tissue

Hydrocephalus An interesting development in research on the nervous system has recently been reported by Millen Woollam and Lamming [23] [24] Hydrocephalus has been produced with an incidence of up to 80% in young rabbits whose mothers were deprived of vitamin A for various periods before mating The young were examined either when born or after being themselves restricted to a deficient diet for some weeks after weaning An example of the resulting hydrocephalus with distension of the lateral ventricle with fluid and narrowing of the brain substance is shown in Plate 16

In view of Mellanby s theory that the nervous lesions in vitamin A deficiency are related to compression by bone careful examinations were made of the vaults and bases of the skulls of the hydrocephalic rabbits Ossification was found to be far from complete as exemplified by the auditory capsule being still almost entirely cartilaginous No evidence was found of compression of the central nervous system by an overgrowth of bone Indeed in the vault of the skull the bony tissues were thinner than usual which is in accordance with the expansion of the calvaria to be expected in congenital hydrocephalus

In seeking to explain the development of the hydrocephalus Millen and his colleagues were first attracted by the possibility that fluid might accumulate in the ventricles because of stenosis of the cerebral aqueduct which prevented its free passage into the subarachnoid space After further investigation however an excessive production of fluid by the choroid plexuses with a secondary distortion of the aqueduct seemed a more probable explanation



Plate 16 Hydrocephalus in rabbits (Millen et al 1953) (1) Coronal section through the head of a normal rabbit (2) Rabbit deficient in vitamin A

These important observations indicate a need for caution against the adoption of any rigid and generalised theory on the interdependence of the bone and nerve lesions in vitamin A deficiency In Mellanby s experiments the normal development of the nerves seems undoubtedly to have been influenced by disorganised growth of the bone In Millen s work the developing bone was forced out of its shape by the expanding brain which

*References p 3*8

was in turn under pressure from the cerebro spinal fluid It seems reasonable to conclude that vitamin A deficiency can have primary effects on nerves bones and on epithelial tissues The exact nature of the lesions sustained by any animal will depend on the interplay of factors such as species age and rate of growth

A list of the various theories advanced at different times by the workers in this complicated field is given in Table 38

TABLE 38

THEORIES ON THE PRODUCTION OR SIGNIFICANCE OF NERVE LESIONS IN VITAMIN A DEFICIENCY

MELLANBY 1935	Epithelial lesions in vitamin A deficiency including xerophthalmia were thought to be *secondary effects of injuries to the* corresponding nerves The inclusion of cereals in the diet appeared to aggravate the injuries (*Rabbits*)
RAO 1936	No correspondence was found between the severity of injuries to the eyes and the nerves Degeneration of the nerves was found even when cereals were excluded from the diet (*Rats rabbits fowls*)
LANE MOORE 1935	Blindness could arise even in the absence of xerophthalmia by injury to the optic nerve through narrowing of the optic foramen Importance of vitamin A deficiency seemed uncertain (*Calves*)
MELLANBY 1937	In vitamin A deficiency overgrowth of bone in the labyrinthine capsule caused pressure on the 8th nerve (*Dogs*)
LANE MOORE 1941	Deficiency of vitamin A caused increased pressure in the cerebrospinal fluid (*Calves*)
MELLANBY 1943	... the interplay between ... modelled and
WOLBACH and BESSEY 1941	In vitamin A deficiency nervous tissues grew more rapidly than bone which caused compression of the brain and herniations (*Rats*)
RIGDON 1952	Nervous lesions in avitaminosis A causing ataxia could not be related to injuries to the bone (*Ducks*)
BLAKEMORE 1950	(*Calves*)
MILLEN 1953	Hydrocephalus can be produced in young animals by avitaminosis A The aqueduct may be stenosed but an excessive production of cerebro spinal fluid seems more important (*Rabbits*)

CHANGES IN CHRONAXIA

Our review of the effects of vitamin A deficiency on nerves may be concluded with brief mention of investigations in France on the influence of the vitamin on the speed of excitation of nerves and muscles These studies have been based on the measurements of chronaxia which is expressed in units based on the time necessary to cause excitation on passing a current twice as strong as the rheobase , which is the minimum current necessary to cause excitation when passed for a long time

Chevallier and Espy [25] noticed that the chronaxia of the motor nerves to the paws of guinea pigs and rats was related to their vitamin A reserves In guinea pigs with adequate reserves of vitamin A values of about 350 σ were found for the extensor muscles of the legs and 200 σ for the flexor The difference between these values was ascribed to subordination and anaesthesia had the effect of increasing chronaxia for the flexor muscles so as to give the same readings as for the extensor muscles for which the chronaxia remained unchanged In guinea pigs without measurable reserves of vitamin A the chronaxia values were only about 200 for both extensor and flexor muscles and were little changed by anaesthesia Similar observations were made on rats pigeons and frogs A further paper [26] reported that in guinea pigs the vitamin A reserves tend to be higher in spring than in autumn and that corresponding changes can be observed in chronaxia A further complication was introduced by the finding that some guinea pigs but not all have surprisingly low reserves of vitamin A (Chap 13)

In later studies by Lecoq Chauchard and Mazoue [27] it was found that when rats were restricted to a diet deficient in vitamin A the chronaxia for the nerves began to fall after 15–17 days while the chronaxia for the muscles rose after prolonged deprivation Changes in chronaxia were also induced by deficiency in other vitamins but when the diet was deficient in vitamin A the abnormal chronaxia could not be corrected by dosing with any vitamin other than vitamin A The theory was developed that deficiencies of the various vitamins affect the acid base balance of the body in different ways and lack of vitamin A was claimed to cause acidosis The addition of sodium bicarbonate to the diet of the deficient animals caused the chronaxia to return to normal but did not cure xerophthalmia When carotene was given to rats deficient in vitamin A the abnormal chronaxia was only corrected after 3 days as compared to one day after dosing with preformed vitamin A [28]

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CHAPTER 28

Defective Reproduction. Changes in the Reticulo Endothelial System. Haematological Abnormalities

DEFECTIVE REPRODUCTION

The effect of vitamin A deficiency on the testes and uterus will be discussed in Chapter 36, which deals with the interrelationships between the vitamin and sex For the present it will suffice to mention that studies in rats have shown that the testes become degenerated and lose their power to form spermatozoa In the uterus the changes due to the oestrus cycle can no longer be demonstrated, except perhaps by special methods of staining, and scales of keratin are found continuously in the vaginal smear In both the testes and uterus the injuries sustained originate in epithelial tissues

Abnormal gestation The cessation of the oestrous cycle in the deficient rat, or at least its masking by the predominance of keratin formation would suggest that fertilisation could occur only with difficulty if at all In agreement with this expectation Parkes and Drummond [1] found that reproduction was completely arrested but their rats had been made very acutely deficient and had stopped growing at body weights under 100 g Complete or partial infertility was also reported by other workers [2] [3] Mason [4], however was later more successful in effectively mating his animals, and in studying the effects of complete or partial deficiency of the vitamin on the course of gestation and delivery

Females which were acutely deficient in vitamin A but which had not reached the stage of developing xerophthalmia often became pregnant when they were mated with normal males The foetuses seldom survived for long however and their deaths were followed by the resorption of the products of their degeneration back into the blood stream The final effect therefore was similar to that seen in deficiency of vitamin E but *post mortem* examination indicated that the pathological sequences were quite different Thus in avitaminosis E the injuries first affected the foetus and were later communicated to the placenta whereas in deficiency of vitamin A this sequence was reversed In rats which might have been deficient in either of the vitamins Mason could easily decide which deficiency had exerted the predominating influence In histological studies of rats which had been deficient

References p 339

in vitamin A he found localised areas of infection leucocytic infiltration and cellular necrosis at the foetal sites

In those acutely deficient animals which succeeded in carrying their young to full term and in others in which pregnancy was prolonged by giving small doses of cod liver oil the maternal injuries were more severe than after resorptions Often the foetuses some dead and some alive could not be expelled after the normal period of 21 days but remained unborn even after 26 days The prolongation of pregnancy and difficult gestation were often associated with excessive uterine bleeding and with the accumulation of foul smelling fluid due to infection Sometimes the mother rat died either before or during delivery

As might have been expected after unduly long gestation periods the foetuses tended to vary greatly in size with some about normal at 3–4 5 g and others overdeveloped at 6–8 g It was concluded that all these abnormalities originated from epithelial injuries and that the deficiency had no ill effects on the ova the mechanism of implantation or the functions of the ova or anterior pituitary Later an extensive investigation by Newton[5] substantially confirmed Mason s findings In this work uncompleted pregnancy was observed much more frequently than difficult labour which was often complicated by infection of the uterus

Congenital malformation We have now to consider the effect of vitamin A deficiency on the anatomy and histology of the foetus In regard to the typical epithelial lesions as seen after birth in adolescent or mature animals little information seems to be available Wilson and Warkany[6] have reported however that in foetuses carried by rats deficient in vitamin A metaplastic keratinisation may be seen in the

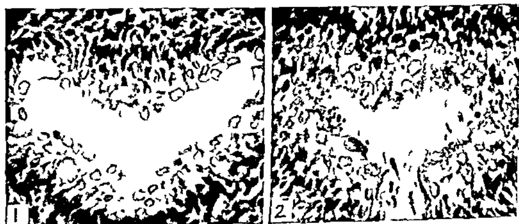


Plate 17 Sections of the urethras of new born rats (1) Offspring of a mother receiving adequate amounts of vitamin A (2) Offspring of a mother deficient in vitamin A Note the keratinising metaplasia in the epithelium (Wilson and Warkany 1947)

genito urinary tract from the 18th day of gestation In epithelial tissues in other parts of the body no keratinisation is observed (Plate 17)

Much more striking lesions however are developed in other types of tissue and may give rise to gross malformations Thus Hale [7] was impressed by instances of pigs being born without eyeballs and later proved that this abnormality was due to deficiency in vitamin A [8, 9] Often anophthalmos or microphthalmos was accompanied by deformities such as accessory ears, harelip and cleft palate subcutaneous cysts and misplaced kidneys Since the gilts were known to be of sound stock the cause of the defects could not be traced to hereditary factors The conclusion seemed inevitable that common congenital deformities such as harelip might arise not from defects in the sperm or ova but from the malnutrition of the developing foetus

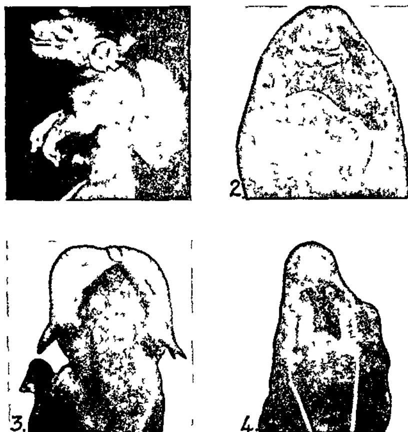


Plate 18 Congenital deformities in piglets produced by sows deficient in vitamin A None of the piglets had eyeballs Associated abnormalities were (1) Subcutaneous cysts on the head and back (2) Double cleft lip (3) Extra ear like growths (4) Cleft palate (Hale 1935)

The fruitful field of research which obviously lay open for the extension of these observations to some small laboratory animals such as the rat,

References p 339

was first entered by Andersen[10] In 25% of the young rats which had been bred from mothers given inadequate doses of vitamin A she observed diaphragmatic hernia The same lesion was occasionally noticed, however, in rats bred from mothers given halibut liver oil While deficiency of vitamin A certainly increased the frequency of the abnormality further work suggested that hereditary factors were also involved[11]

Extensive detailed investigations were next undertaken by Warkany and his colleagues Congenital malformations of the rat s eye were first de scribed[12, 13] Out of 140 females which were raised and mated on a diet low in vitamin A and were then completely deprived of the vitamin during pregnancy only 7 carried their litters to full term The eyes of the young were *invariably abnormal, with the replacement of the vitreous body by* a fibrous retrolenticular membrane as the most constant finding In addition there were frequently colobomas eversion abnormal structure and folding of the retina, rudimentary development of the iris and ocular chambers, defects of the cornea and conjunctival sack, and lack of fusion of the lids The last abnormality led to a condition of "open eyes" which could readily be recognised by superficial inspection Similar observations were also made by Jackson and Kinsey[14] who also studied the level of vitamin A in the blood plasma of the mother rats Levels of less than 12 i u per 100 ml were found in those animals whose young had ocular deformities (Plates 19 and 20)

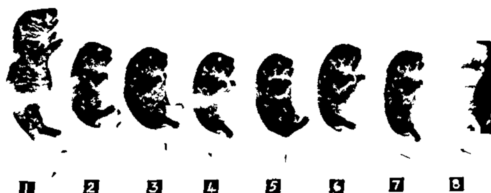


Plate 19 Congenital deformities in rats from a mother deficient in vitamin A (1) Normal newborn rat (2–8) Foetuses removed on the 22nd day of pregnancy from the uterus of a mother deficient in vitamin A "Open eye" can be seen in Nos 2 and 4 In No 4 this abnormality is accompanied by cutaneous haemorrhage (Warkany and Roth 1948)

Later Warkany and Roth[15] tried to increase the numbers of abnormal young available for study by adjusting the allowance of vitamin A but found difficulty in choosing a dose which would at the same time allow a high incidence of fertility and a low incidence of normal young An allowance of 10–25 μg of carotene every tenth day was found to be most suitable

A routine was devised in which the eyes of representative rats in each litt were examined and inspection for injuries in other parts of the body we only undertaken when ocular lesions were found

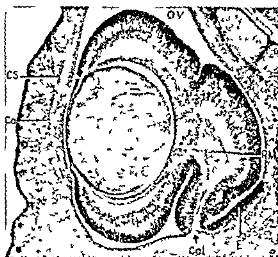


Plate 20 Section through the eye of an abnormal newborn rat produced by a mothe deficient in vitamin A Col = coloboma E = eversion of retina RM = fibrou retrolenticular membrane connected with extra ocular mesoderm OV = space o optic vesicle CO = conjunctiva CS = conjunctival space (Warkany and Schraffer berger 1946)

Extensive studies were next undertaken by Wilson and Warkany [16] o the genito urinary tract Abnormalities were found in this region in abou 75% of the foetal or newborn rats from deficient mothers Anomalies o paraplasia resulting from aberrant embryonic processes were occasionall observed in the form of fused kidneys or stenosis of the ureters or of th homologous genital ducts Aplasias or anomalies characterised by a complet inability of structures to develop were seen in failure of the male accessor sex organs to appear and in the lack of vaginal development in mos females Malformations of hypoplasia resulting from the arrest or retardatio of an embryonic process whether progressive or regressive were howeve the most common forms of lesion Thus the partitioning of the cloaca th appearance of the Mullerian ducts and the differentiation of the urogenita sinus were all delayed The kidneys failed to assume their normal positior the ureteric openings were ectopic the caudalward growth of the Mullerian glands was incomplete the mesonephric glands were poorly differentiated and the testes failed to descend Hypoplasia in the special sense of a slowin down or failure in the regressive processes of development was observed i the persistence of heterogenous genital ducts in both sexes and in a retentio of the urethral plate No instances of hyperplastic malformations charac

References p 339

terised by over growth or over-activity of an embryonic process, were encountered (Plate 21)

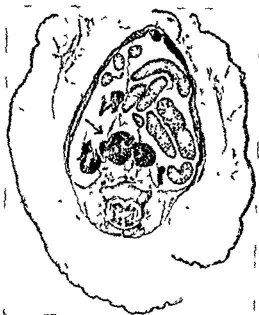


Plate 21 Section through the body of the offspring of a rat deficient in vitamin A Note that the kidneys are fused together (Warkany [28])

Cardiac abnormalities now remained for investigation [17]. The rats selected for study had already been found abnormal by the detection of ocular or uro-genital lesions, which were about equally common Out of 64 of such animals there were 22 instances in which the interventricular septum of the



Plate 22 Section of the heart of an abnormal young rat Note that there is no division between the right (1) and left (2) ventricles (Wilson and Warkany [30])

heart failed to close and 22 instances of deformity of the aortic arch, with a combination of these abnormalities in 16 instances Myocardial development was often retarded, with the result that the wall of the heart had a highly trabeculated, spongy appearance All these cardiovascular effects were seen most often in young foetuses They were thus usually associated with early foetal death, although not necessarily its cause (Plate 22 and Fig 22)



Fig 22 The effect of vitamin A deficiency on the aortic arches of the rat foetus (Wilson and Warkany 1950) (1) Full hypothetical embryonic pattern This complete system is never actually attained during any stage of the development of the foetus (2) Normal development of the arches as found after the 16th day of gestation (3) One of the numerous forms of abnormality which were observed in vitamin A deficiency In this instance there was a double aortic arch Other abnormalities included right aortic arch retro oesophageal subclavian artery absence and other variations in the ductus arteriosus and absence of a pulmonary artery

From all these studies we can safely conclude that deficiency of vitamin A can be a potent factor in the production of congenital abnormalities It is less clear, however, to what extent the foetal abnormalities found in avitaminosis A are specific to deficiency of this vitamin An excellent review by Giroud [25] has recently made it plain that congenital abnormalities can result from deficiencies of several other nutrients Moreover abnormalities may be caused by various other forms of stress, such as infections, poisons and exposure to x-rays It is interesting to note that congenital cleft palates, one of the effects of maternal vitamin A deficiency, may also be produced by deficiency of riboflavin

Changes in the Reticulo-Endothelial System

The reticulo-endothelial system may perhaps be considered, at least in a histological sense, as being as far removed as possible from the epithelial tissues in which the effects of vitamin A deficiency were originally studied.

References p 339

There have been several reports, however, that abnormalities may readily be detected Thus in deficient rats De Ruyter[18] noticed changes in the spleen and in the Kupffer cells of the liver. When trypan blue was injected into deficient animals it was only poorly absorbed by the Kupffer cells In the Malpighian corpuscles of the spleen and in the lymph glands free histiocytic elements and plasma cells replaced small lymphocytes, which were greatly reduced in number

An independent study by Frank[19] soon confirmed the abnormality in the Kupffer cells A comparison of the livers of deficient and normal rats was made after the injection of lithium carmine In the normal animals the Kupffer cells were normal in number and fine in shape Their nuclei stained strongly with basic dyes, and their narrow protoplasmic margins contained numerous small granules of lithium carmine In contrast the Kupffer cells of the deficient animals were not only more numerous, but were much larger. The nuclei stained poorly, and in their distended protoplasm the grains of lithium carmine often appeared to be fused together. Frank commented that similar changes had previously been reported as a general indication of the degeneration of the Kupffer cells He suggested that this degeneration might provide a reason for the reduced resistance to infections which is typical of vitamin A deficiency.

Very similar observations were also described by Uotila and Simola[20]. In both rats and guinea pigs vitamin A deficiency caused a hyperplasia of the reticulo-endothelial system. The Kupffer cells were increased both in size and in number The red pulp of the spleen was hypertrophied, and its lymphoid tissue atrophied

Before leaving this topic we may recall that the Kupffer cells are important centres for the concentration of vitamin A (Chap. 19) No similar concentrations, however, have been found in other parts of the reticulo-endothelial system.

Haematological Abnormalities

In contrast to the general agreements between reports on the effect of vitamin A deficiency on the reticulo-endothelial system our information on haematological abnormalities seems both inadequate and inconsistent

Sure, Kik and Walker[21] found that in rats suffering from the early stages of deficiency there was a suggestion of anaemia, characterised by a reduction in either haemoglobin or erythrocytes After xerophthalmia had developed, however, the figures for both haemoglobin and erythrocytes were raised, probably as the result of anhydraemia. No claim was made that these results should be accepted as conclusive. Observations by Frank[22], also made upon rats, indicated reduced values for haemoglobin and erythrocytes, and thus

agreed with those of the American workers for the early stages of deficiency

Rather more extensive observations have been made on the effects of deficiency on the white cells In his rats Frank found that the total leucocyte count was reduced with an increase in the ratio between neutrophiles (or polymorphonuclears) and lymphocytes In blood from two human infants with xerophthalmia the same changed ratio was found but the total white cell count was increased Crimm and Short [23] examined rats which were stated to be suffering from early deficiency of vitamin A but which were found by chemical examination to be still retaining considerable reserves of the vitamin in their livers Their main observation was a right shift of the Arneth index Thus there was a delay in the rate of maturation of the neutrophiles as indicated by the preponderance of senile forms No change occurred however in the gross number of all stages of neutrophiles Injections of foreign protein into the deficient animals caused an unduly long rise in the leucocyte count which suggests that the reticulo endothelial system was working inefficiently

From experiments with rats Abbott and Ahman [24] concluded that prolonged deficiency caused a decrease in the neutrophile count with an increase in the number of juvenile cells and an increase in the ratio of large to small lymphocytes The first effect of deficiency however appears to have been a marked rise in neutrophiles associated with an equally steep fall in lymphocytes during a temporary cure by the administration of carotene these tendencies were completely reversed In human subjects suspected to be suffering from vitamin A deficiency as indicated by conjunctivitis and dryness of the skin Abbott Ahman and Overstreet [25] recorded much the same picture as they had found after prolonged deficiency in rats Heavy doses of vitamin A improved the condition of the subjects and brought the differential leucocyte count within the normal range By the prolonged and heavy dosing of normal subjects Crimm and Short [26] induced a left shift in the Arneth index indicative of a preponderance of young neutrophiles

In both rats and human infants Frank [22] found that vitamin A deficiency interfered with the blood clotting mechanism with increases in fibrinogen and in the blood coagulation time Anagnostu [27] reported that in rats kept for 5–7 weeks on a deficient diet the thrombocyte count fell by 31–69% A diminution in the cell content of the bone marrow was also observed which particularly affected the eosinophilic cells The development of megakaryocytes which are presumed to be the parent cells of thrombocytes was interrupted The strong evidence showing that massive doses of vitamin A greatly prolong the clotting time of the blood will be discussed in Chapter 28

References p 339

From all the above evidence it may be concluded that changes in the blood picture, and particularly in the number and distribution of the white cells, occur in animals deprived of vitamin A. It remains to be decided, however, how far these changes are due to a primary deficiency of the vitamin, rather than to the resulting inanition and secondary infections. Inconsistencies between the reports from different workers, and even from the same worker regarding different stages of his experiments, suggest that the findings which have so far been reported must be interpreted with caution.

General Conclusions

In conclusion we may refer once more to the diversity of the lesions which may be suffered in vitamin A deficiency. To take an extreme example it might be difficult to recognise that a deficient rat and a deficient pig were both suffering from avitaminosis A if their dietary history were unknown. Thus the rat, if showing the most typical signs of deficiency in this animal, would exhibit unmistakable xerophthalmia associated eventually with extreme emaciation. In contrast the eyes and body weight of the pig might be little affected, but the animal would be found to be unable to stand on its hind legs.

It is also clear that the lesions sustained in vitamin A deficiency vary greatly in their specificity. Thus xerophthalmia and the disorganised overgrowth of bones are highly characteristic signs of deficiency, which cannot be produced by any other common diseases. Localised xerosis of the mucous membranes, however, may occur as a secondary effect of infection in a form substantially the same as in avitaminosis A. Pneumonia, enteritis and infections of the urinary tract will present much the same final picture whether they originate from deficiency of vitamin A or from some other cause. Without a knowledge that rats had been receiving a diet deficient in vitamin it would be difficult to decide on the cause of the enteritis which often kills them before xerophthalmia has time to develop.

We cannot, therefore, entirely dismiss the possibility that the human subject may sometimes suffer from avitaminosis A as manifested by its general rather than by its specific signs. Further discussion on this topic is included in Chap. 39.

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CHAPTER 28

Hypervitaminosis A

Vitamin A shares with vitamin D the property of causing characteristic injuries when it is given in very large excess. These lesions are important as clues to the mode of action of the vitamin.

Poisoning by cod-liver oil. In 1920 the Japanese worker Suzuki[1] reported that great excess of cod-liver oil was injurious to rats He considered that the toxicity was due to the unpleasant smell and inferior quality of the sample under investigation Subsequent work, however, has shown that the more highly unsaturated fatty acids of cod-liver oil may be injurious in various animals, even when they are given in the form of fresh oil Vitamin E is destroyed or its action is opposed, and various lesions related to vitamin E deficiency are incurred This interesting subject, however, obviously falls beyond the scope of this book.

It may be mentioned, however, that in 1927 Hartwell[2] noticed that when pregnant rats were given diets containing large amounts of cod-liver oil they had difficulty in giving birth to their litters, and usually died from profuse uterine haemorrhage. Hartwell suggested, with surprising insight at that time, that the cod-liver oil destroyed vitamin E. It still seems possible, however, that the haemorrhage may have been due to the large amounts of vitamin A in the cod-liver oil (see below).

Vitamin A concentrates. The first evidence that vitamin A itself was toxic was put forward in 1925 by Takahashi and his colleagues[3] Although the isolation of the vitamin had been claimed only crude concentrates were actually available. When daily doses 10,000 greater than the minimum necessary for growth were given by mouth to rats or mice death usually occurred after about 2 weeks. The symptoms observed included alopecia of the head, paralysis of the hind legs and progressive emaciation. Autopsy revealed fatty degeneration of the liver, kidneys and heart. In the digestive tract there was hyperaemia with haemorrhage, and in the lungs these injuries were often accompanied by nodular caseation By substituting injections of the concentrates for oral doses injuries were

more rapidly produced Paralysis of the hind legs was seen only 15 minutes after the concentrates had been injected, and was followed by general cramp and death, sometimes within an hour

The ill effect of vitamin A concentrates in causing loss of weight and skin lesions was confirmed by Harris and Moore[4] and others[5-8] Chevallier, Cornil and Chabre[9] suggested that the lesions seen in hypervitaminosis A resembled those seen in scurvy

In 1933 when vitamin A concentrates were approaching a state of purity, another striking lesion was observed Collazo and Sanchez Rodriguez[10] noticed that the bones of rats given excessive doses of concentrates became extremely fragile Even the limited movements possible in caged rats caused fractures of large leg bones The same injuries were described independently by Bomskov and Seeman[11] and were confirmed by many other workers[12-15]. Davies and Moore[16] noticed that the broken ends of the bone were sometimes ankylosed together, with the formation of large irregular calluses

In 1943 Rodahl and Moore[17] drew attention to heavy internal haemorrhage as a cause of sudden death in rats given excessive amounts of rich sources of vitamin A These haemorrhages often occurred under the skin, but were also found in various other sites Thus in one animal the pericardium was engorged with blood Such heavy haemorrhages were different in form and intensity from the diffuse bleeding at membranes which had already been reported in non pregnant animals In their intensity, at least, they resembled the uterine haemorrhages reported by Hartwell[2] in pregnant rats given excessive cod liver oil

Poisoning by pure vitamin A All the above findings left no doubt that excessive amounts of rich sources of vitamin A were toxic, but it was less certain whether the poisoning was due to the vitamin itself As already mentioned the glycerides of cod liver oil may sometimes cause injuries Agduhr[18] reported the ill effects of cod-liver oil on the musculature of various animals and pointed out that the oil was still injurious after its vitamin A had been destroyed Yamamoto[19] and later Yoshida[20] concluded that the glyceride fraction, and not vitamin A, was responsible for the adverse effects of many marine oils on rats Matsuoka[21] found that cramps followed the injection of concentrates which had been freed from vitamin A by hydrogenation or oxidation but that a distillate containing vitamin A did not cause cramps In preparing crystalline vitamin A derivatives Hamano[22] found that toxic substances accompanied the vitamin into the unsaponifiable fraction, but claimed that they could be separated from it by their lower solubility in methanol Vedder and Rosenberg[14] found no close relationship between vitamin A potency and toxicity in distillates prepared from jewfish liver oils

References p 350

The evidence at this point suggested that substances associated with vitamin A rather than the vitamin itself were the cause of toxicity Tests with the pure vitamin were therefore needed to decide whether it was toxic at all If it was toxic it had then to be decided which of the lesions which had already been reported were due to the vitamin itself and which were caused by other components of liver oils

In 1945 Moore and Wang[23] examined the toxicity of a specimen of crystalline vitamin A acetate having $E_{1\%}^{1cm}$ at 328 mμ = 1500 When young rats were given the vitamin A mixed with a portion of their diet in daily doses of 25 000 or 40 000 i u a limping gait was noticed in some of the animals after about a week and eventually in all the animals Growth was checked and there was pronounced exophthalmus Death usually occurred after about 3 weeks X ray photographs showed fractures of the bones (Plate 23) and at autopsy profuse subcutaneous and intramuscular hae morrhages were usually found Analyses of the bones for the percentage of

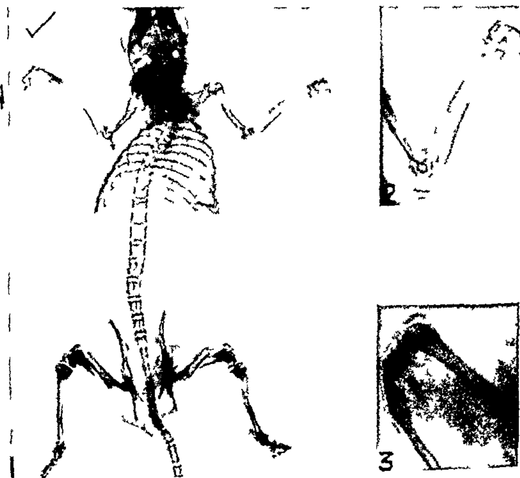


Plate 23 Skeletal fractures caused by hypervitaminosis A in the rat (Moore and Wang 1945) (1) Fractured bones include both femurs (2) Fracture of radius (3) Fracture of tibia and fibula followed by healing and callus formation

ash indicated no gross abnormality in their mineral metabolism It appeared, therefore that the fault lay in the formation or destruction of the matrix

Injections of single large doses of vitamin A into young rats had no serious ill effects Sometimes short periods of cramp were observed The postures of the animals however often gave the impression that they were merely trying to avoid discomfort One animal was injected with 400 000 i u of vitamin A alcohol prepared from the crystalline acetate but showed no evidence of cramp Two out of three rats which were injected with a crude concentrate of fish liver oil developed cramps within an hour but later recovered

When pregnant rats were given daily doses of 40 000 i u of vitamin A acetate starting within 10 days after coitus about half the animals failed to deliver their litters and died from severe uterine haemorrhage In most of the remaining animals implantation failed or the foetuses were resorbed at an early stage Instances of uterine haemorrhage were also observed in rats given excess of halibut liver oil or of cod liver oil It appeared therefore that bone lesions and haemorrhages were specific effects of overdosage with vitamin A, but that other injuries such as cramping were not caused by the vitamin

The incidence of the injuries appeared to be influenced by the age of the rat Adult rats did not have fractured bones but they were not immune from severe haemorrhages

Paveck Herbst and Elvehjem [24] found that great excess of vitamin A was equally toxic to rats whether it was given as the crystalline vitamin or as halibut liver oil Pathological telang livers from cattle which were found to be very rich in vitamin A were toxic in proportion to their vitamin A contents

The resemblance to scurvy As early as 1922 Mouriquand and Michel [25] claimed that cod liver oil antagonised vitamin C in guinea pigs causing scurvy even when lemon juice was given As already mentioned Chevallier and his colleagues [9] also suggested that the injuries produced by excess of vitamin A resembled scurvy Collett and Eriksen [26] later supported Mouriquand s conclusion with regard to cod liver oil but found that a moderate excess of vitamins A and D had no injurious effect Although normally the rat is not liable to scurvy since it is able to synthesise ascorbic acid Vedder and Rosenberg [14] pointed out that the symptoms caused in their animals by excess of fish liver oils resembled this disease

The profuse intramuscular and subcutaneous haemorrhage observed by Moore and Wang [23] brought the picture in hypervitaminosis A even nearer

References p 350

to that seen in scurvy. Accordi...
or between the muscle planes...
subjects, while similar haemorrhages are also frequent in experimental scurvy in the guinea pig

In regard to the skeletal lesions the resemblance between hypervitaminosis A and scurvy is less close In scurvy the human infant is particularly vulnerable to separation of the epiphysis, and to fractures near the growing end of the bone These injuries may sometimes be seen in rats, but fractures near the middle of the long bones are more common

The biochemical evidence that hypervitaminosis A is a form of scurvy is also unconvincing Moore and Wang [23] found no differences between the ascorbic acid contents of the liver adrenals and urine of normal and hypervitaminotic rats According to Rodahl [28], however, ascorbic acid was reduced in the livers and blood serum of hypervitaminotic guinea pigs Morehouse, Guerrant and Dutcher [29] confirmed Rodahl's finding on the liver of the hypervitaminotic rat, but Eeg Larsen and Pihl [30] considered that the decline in ascorbic acid was due merely to a reduced consumption of food

Opinions have also differed on the value of ascorbic acid in counteracting the effect of excessive doses of vitamin A Vedder and Rosenberg [14] reported that ascorbic acid protected rats which were overdosed with jewfish liver oil, and Rodahl [31] reached similar conclusions for other sources of the vitamin On the other hand Moore and his colleagues [23, 32], Morehouse, Guerrant and Dutcher [29] and Eeg-Larsen and Pihl [30] all found that ascorbic acid was quite ineffective in counteracting the effects of hypervitaminosis A

Induced vitamin K deficiency The severe haemorrhages noticed by Moore and Wang [23] in adult hypervitaminotic rats had occurred in various sites Thus one animal which had been overdosed with halibut-liver oil for 17 days died suddenly from massive haemorrhage under the capsule of the spleen, which became detached and greatly distended with blood Another animal suddenly became paralysed after 18 days of dosing, without any premonitory loss of weight and at autopsy was found to have severe haemorrhages in and around the bladder, in the pectoral muscles and inside the cranial cavity at the base of the skull

The immediate cause of these sudden haemorrhages was made clear by Light, Alscher and Frey [33] When rats were dosed with about 18 000 i u of vitamin A daily for 10 days the delayed clotting time of their blood plasma indicated a marked hypoprothrombinaemia When the daily dose was increased to about 40,000 i u many of the animals died from cerebral haemorrhage The hypoprothrombinaemia was prevented if the excessive intake of vitamin A was accompanied by daily doses of 25 μg of synthetic vitamin K_1

The occurrence of secondary vitamin K deficiency as a symptom of hyper vitaminosis A was confirmed by Walker, Eylenberg and Moore [32] The administration of artificial vitamin K substitute Synkavit which is the tetrasodium salt of the diphosphoric ester of 2 methyl 1 4 naphthoquinone prevented the hypoprothrombinaemia and reduced the incidence of haemorrhage Bone lesions however were not prevented by the Synkavit The administration of dicoumarol to rats not given excess of vitamin A moreover produced no skeletal lesions to accompany the well known hypoprothrombinaemia caused by the anticoagulant The injuries in the blood and bones therefore did not appear to be closely interrelated

Walker and her colleagues also noticed that the blood of hypervitaminotic rats was thin and watery The average plasma cell ratio for animals given excess of vitamin A without vitamin K was 2 2 as compared with 1 1 for control animals This change in the haematocrit was observed even in the blood of rats which has no haemorrhages Possibly these findings may be related to a claim by Poumeau Delille [34] that toxic doses of vitamin A induce a severe erythroblastic anaemia in rats

Epithelial abnormalities Domagk and von Dobeneck [35] found that the administration to rats and mice of massive over doses of vitamin A interfered with the formation of keratin in epithelial tissues In sites where thin layers of unnucleated keratin would normally be seen several layers of immature cells were rapidly proliferated These changes were plainly seen in the anterior part of the rats stomach

Studer and Frey [36] studied the effect of massive overdosing with synthetic vitamin A acetate on the skin of rats On the back the epidermis increased to $1\frac{1}{2}$–3 times its normal thickness during the first 12 days of dosing after which it returned towards normal In constrast the skin in vitamin A deficiency was abnormally thin

The effect of massive doses of vitamin A in counteracting the keratinising effects of oestrogens on the vaginal epithelium is decribed in Chapter 36

The reticulo endothelial system and kidneys In their hypervitaminotic mice Domagk and von Dobeneck [35] noticed a striking deposition of fat in the Kupffer cells of the liver and in the pulp cells of the spleen Similar deposits were seen in the endothelial cells of the glomeruli of the kidney and between the urinary channels in the cortex of this organ Fat also appeared in the endothelium of the lung capillaries It was concluded from these observations that vitamin A must play an important part in metabolism of fat

According to Laubmann [37] the injuries to the reticulo endothelial system in hypervitaminotic rats were accompanied by glomerulonephrosis and calcification of the kidneys Later Noetzel [38] confirmed the deposition of fat in

References p 350

the reticulo-endothelial system, and described the renal injuries as necrotic nephrosis.

Bone formation. The effects of deficiency and excess of vitamin A on the bone growth were compared by Wolbach [39]. In rats the administration of greatly excessive doses of the vitamin caused acceleration of the growth of the epiphyseal cartilage cells. Maturation and the remodelling processes accompanying growth also accelerated. The growth sequences in the epiphyseal cells were also accelerated in dogs, in which hyperaesthesia and exophthalmos were the outward signs of hypervitaminosis. Wolbach *et al.* 40, 41 found that the bone changes in hypervitaminosis occurred so regularly in rats that they could be made the basis of a rapid biological test, suitable for application to highly potent artificial forms of the vitamin.

The acceleration of bone growth suggested that the excess of vitamin A might exert its action by stimulating the anterior pituitary gland. Wolbach and Maddock [42] found that the bones of hypophysectomised rats were affected even more rapidly than those of normal animals by excess of vitamin A.

In contrast to the finding in mammals Rigdon, Rude and Bieri [43] could detect no abnormalities in the bones of ducklings which were given excessive doses of vitamin A for 7 weeks. Wolbach and his colleagues, however, found that hypervitaminosis A caused disturbances in bone growth both in chicks and in ducks [44]. The abnormalities were completely parallel with those seen in mammals when the different growth pattern in the epiphyseal cartilage in birds was taken into account.

Hydrocephalus. In human infants it has been found that massive overdosage with vitamin A may cause a transient bulging of the fontanel, associated with increased pressure in the cerebro-spinal fluid. This interesting phenomenon will be discussed in Chapter 33

Hypervitaminosis A and tissue culture. Important studies of the effects of excess of vitamin A on the growth of tissues have recently been made by Fell and Mellanby using the elegant methods of tissue culture. Their interest was first directed towards the formation of bones, and was later turned to the growth of skin.

In their investigations on bone formation [45] the long bones of late mouse foetuses were cultivated on watch glasses in fowl plasma supplemented with an extract of chick embryo. When pure vitamin A acetate was added to the culture medium at levels of 1000–3000 i.u. per 100 ml the normal progress of the bones was arrested. Within 3 days the matrix of the terminal cartilage softened, shrank, and finally disappeared, although the cartilage cells appeared to be normal. In the shaft the cartilage was rapidly replaced by

marrow tissue The bone was largely resorbed, but the soft tissues surrounding the explant grew as vigorously as in controls cultivated in the normal medium Similar results were obtained in the cultivation of the limb-bone rudiments from 5–6 day-old chick embryos [48]

These findings indicated clearly that the excess of vitamin A interfered with bone formation The abrupt arrest of the growth of bone caused by the excess of vitamin, however was obviously not a complete parallel to the great acceleration of growth seen in the intact animal

The work which followed on the effect of vitamin A on the growth of skin [47] was perhaps even more instructive Explants of ectoderm from the trunk and limbs of 6–7 day old embryonic chicks were cultivated *in vitro* under the same conditions as had been followed in the previous work When cultures were made in medium prepared with normal fowl plasma the explants behaved normally in forming keratinising squamous epithelium In the culture with plasma containing added vitamin A however keratinisation was suppressed The ectoderm differentiated into mucus secreting epithelium, often ciliated similar to that of normal nasal mucosa Large goblet cells were formed If the explants were now transferred to the medium without added vitamin A the differentiation into secretory cells was first accelerated, but after some days squamous keratinising epithelium was formed underneath the secretory layer Eventually the secretory cells were shed off so as to leave normally keratinised ectoderm

Congenital anomalies The frequency of fatal haemorrhage in pregnant rats which are given excessive doses of vitamin A has already been mentioned Cohlan [48, 49] extended the study of the effect of hypervitaminosis A on pregnancy by dosing female rats with 35 000 I U of vitamin A between the 2nd and 16th day after mating and then killing



Plate 24 Anencephaly in the offspring of a rat given excessive doses of vitamin A (Cohlan 1953)

them after 20 days As the result of this treatment the skulls and brains of about half the offspring were found to be grossly abnormal The most common defect was anencephaly, with an extrusion of the brain to the external

References p 350

surface of the head (Plate 24) Other sporadic abnormalities included macroglossia, harelip, cleft palate, gross defects in eye development and hydrocephalus

Cohlan's interesting findings were confirmed in France by Giroud and Martinet [50-52] who studied the effect of the time of overdosing on the lesions sustained When doses of 60,000 i u daily were given on different days of pregnancy the following observations were made

Overdosing on days	*Effects*
5–7	Usually resorption of foetuses 8% of survivors had anencephaly and a few cleft palates
8–10	Anencephaly in 53% of foetuses Some *spina bifida* anophthalmia microphthalmia a few cleft palates
11–13	Cleft palates in 92% of foetuses A few cataracts and 'open eyes'
14–16	Cleft palates in 49% of foetuses A few cataracts
18–20	Cataracts only

The main ill effects of hypervitaminosis, arranged according to the earliness of the overdosing, were therefore, 1. Resorption of foetuses, 2 Anencephaly, 3 Cleft palates, 4 Cataracts

Hypervitaminosis A compared with avitaminosis

Soon after the discovery that very high doses of vitamin D were toxic it was realised that the injuries were caused by an uncontrolled over emphasis of the vitamin's normal action In rickets the level of calcium in the blood is lowered and the calcification of growing bones is checked Normal doses of vitamin D correct these faults, but toxic doses raise the blood calcium above its normal level, and cause widespread calcification in the kidneys aorta and many other sites other than the growing end of the bone A review of the foregoing evidence suggests that at least some of the toxic effects of vitamin A are likewise caused by an overemphasis of the vitamin's normal activity Other injuries seem to be very similar, however, irrespective of whether they are caused by deficiency or by excess of the vitamin

In Table 39 the lesions sustained by various organs and tissues in avitaminosis A and hypervitaminosis A are compared More knowledge must obviously be accumulated before the significance of all the similarities and differences can be fully appreciated but already some points are clear Thus in avitaminosis the columnar and secretory cells of membranes tend to be replaced by keratinished, squamous material In hypervitaminosis membranes which are normally keratinished are changed in the reverse direction by the formation of mucus secreting cells In both deficiency and excess bone formation is abnormal, but the lesions differ considerably In both conditions

TABLE 39

COMPARISON OF THE LESIONS CAUSED BY DEFICIENCY OR EXCESS OF VITAMIN A

Organ or tissue	*Avitaminosis A*	*Hypervitaminosis A*
Eyes	Xerophthalmia	Exophthalmia
Membranes	Keratinisation in mucous membranes	Mucous cell formation in keratinised membranes
Bones	Defective modelling and cancellous structure	Softening and fractures
Reticulo-endothelial system	Swelling of Kupffer cells	Deposition of fat in Kupffer cells and spleen
Blood	Possibly slightly increased clotting time	Haemorrhage associated with greatly increased clotting time
Reproduction	Resorption or congenital abnormalities	Resorption or congenital abnormalities
Skin	Thinning	Temporary thickening

the reticulo endothelial system is affected, with a swelling of the Kupffer cells in avitaminosis and fat deposition in hypervitaminosis The congenital abnormalities produced in the young of pregnant rats seem to be rather similar whether they are caused by deficiency or by excess of the vitamin We may recall however, that the question of the specificity of congenital abnormalities has already been raised (Chap 27)

Toleration of vitamin A by the liver It is an interesting point that the liver can continue to function normally even when it contains enormous accumulations of vitamin A In the present chapter we have seen that the more serious injuries in hypervitaminosis A occur in sites other than the liver We have learnt from Chapter 19, moreover, that the concentrations of vitamin A in these sites are usually only a small fraction of the concentration in the liver Presumably the liver is able to detoxicate the vitamin, possibly by esterification. To cause damage to sensitive organs, therefore, the rate of dosing must overwhelm the capacity of the liver to absorb and detoxicate the vitamin It is intriguing to think that animals with high reserves of vitamin A might be in danger of poisoning themselves if they were able to eat their own livers

The story of the occurrence of hypervitaminosis A in humans as the result of eating polar bear's liver is included in Chapter 33

References p 350

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PART VII
VITAMIN A IN THE HUMAN

CHAPTER 29

The Vitamin A Status in Normal Health, and in Experimental Deficiency

In historical development our knowledge of the ill effects of vitamin A deficiency in human subjects is of great antiquity Chemical research on the vitamin which was an indispensable preliminary to the quantitative studies of the requirements of the body for the maintenance of health came at a much later period Investigations have inevitably followed in which chemical estimations of the vitamin have been used in the study of clinical avitaminosis Another modern development has been the confirmation of suspected deficiency by measurements of the speed of dark adaptation

It will be obvious that a strictly chronological account of the study of avitaminosis A would have to be interrupted by descriptions of the various chemical and physiological aids to diagnosis As a simpler plan therefore we may deal first with our knowledge of the vitamin A status in healthy subjects and in volunteers subjected to experimental deficiency We can then go back in time to discuss the early clinical reports of xerophthalmia and other ill effects of avitaminosis

The first of this group of chapters will deal mainly with The Vitamin A Status in Normal Health and in Experimental Deficiency It will collect together our extensive knowledge of the amounts of vitamin which are normally present in the body both as reserves in the liver and on call in the blood plasma This chapter will also discuss the effect on the vitamin A status as measured chemically or by dark adaptation of the restriction of healthy volunteers to a diet deficient in the vitamin

The scope of our second chapter will be The Dietetics of Vitamin A including Requirements and Sources Assessments will be made of the allowances of vitamin A or provitamins necessary to maintain health as deduced from observations of the cure of experimental deficiency or from other evidence The topics covered will include the contributions which can be made towards the total daily requirement by various foods and the effect of cooking and other treatments on their vitamin A activity

Thirdly we shall deal with Clinical Observations on Vitamin A De

References p 372

ficiency". An account will first be given of outbreaks of xerophthalmia and night blindness, which can be related with certainty to deficiency of vitamin A. We shall see that these diseases may be caused simply by dietary deficiency, or may sometimes be induced by other factors, such as poor intestinal absorption Evidence that certain skin diseases can be ascribed to vitamin A deficiency will follow, although this relationship seems rather less certain and specific than the close association between deficiency and xerophthalmia Diseases which are caused simply by a dietary deficiency of vitamin A will again have to be contrasted with certain other forms of disease, in which abnormalities in the metabolism of the vitamin are suspected

Our fourth chapter will be concerned with ' Vitamin A in Diseases not attributed to Vitamin A Deficiency". Its purpose will be to review the wealth of evidence on the levels of vitamin A and carotenoids found in the human body in all forms of disease. It will be seen, for example, that the liver reserves of vitamin A are often reduced to vanishing point in certain common diseases, possibly with danger of a secondary deficiency of vitamin A The remarkable excretion of vitamin A in the urine, which is characteristic of some diseases, will be discussed.

The Vitamin A Contents of the Body in Health

Studies by chemical methods on the vitamin A status of healthy human subjects have been made by examinations either of liver or of blood plasma Specimens of liver can seldom be obtained conveniently except at *post mortem*, but when death has been sudden the information provided usually gives a reliable indication of the vitamin A status as it was during life. Estimations on plasma will tell us with certainty only about the amounts of vitamin A and carotenoids which are circulating in the blood stream, and caution will be needed before deductions are made regarding the extent of the body's reserves of vitamin A and the adequacy of the diet In individual cases outstandingly low values for vitamin A, either in the blood or in the liver, will rouse our suspicions that the vitamin A status has been inadequate The range of values consistent with health, however, is so wide that slight individual variations above or below the average mean nothing. In order to obtain useful information on the effect of the diet, or some other factor, on the vitamin A status it is necessary to compare data from large groups.

In the human, as in other higher animals, the liver usually contains much larger amounts of vitamin A than are found in any other part of the body. In early years, when only crude means of measurement were available, estimations on liver were therefore much less difficult than estimations

on blood For this reason surveys were commenced on liver specimens long before accurate estimations on blood were possible To a large extent moreover studies on liver were discontinued when more accurate estimations on blood became possible It seems a fair statement that most liver studies were made with the Lovibond Tintometer before 1939 and that most studies on blood were made with photoelectric apparatus from 1939 onwards Studies on the liver reserves of vitamin A under post war conditions and with the use of modern apparatus certainly seem desirable The accuracy of the tintometer for liver estimations however need not be unduly disparaged

Individual variation in liver reserves

It may be helpful before comparing liver reserves of vitamin A in different parts of the world to study the variation to be expected between healthy individuals in the same country For this purpose the values obtained by the author [1] for a group of 40 cases of accidental death are presented in graphic form (Fig 23) The subjects were all aged 15–59 mostly males and had died between the years 1931–35 within 7 days of their accidents It will be seen that the reserves when arranged in ascending order of magnitude produce a fairly smooth curve up to about 400 i u per g The various ledges as at 180 and 300 i u per g are merely due to the Lovibond matching being made at set intervals rather than on a continuous scale

This wide variation and the absence of any obvious mode makes it difficult to apply conventional statistical methods to compare data for liver reserves of vitamin A obtained on different groups of subjects A simple average can sometimes conceal interesting information because of the undue influence of very high values Thus the effect on the average of several very low values to which our main interest may be attached may be counter balanced by a single very high value In the data given the single top value of 1500 i u per g was about equivalent to the sum of the lowest 16 values

Some special method had to be devised therefore for the comparison of liver reserves of vitamin A between different groups of subjects It occurred to the author that the best plan admittedly imperfect was to first array the data for each group in ascending order and then to divide the group up into three sub-groups For each sub group the average could then be calculated and the three averages low medium and high could be quoted as a summary of findings for the group When it was desired to condense the results still further it appeared that the median was the most significant single value It was taken however not as the individual central value but as the average of the middle sub groups For the data given in Fig 23 the averages were calculated as 75 (low) 220 (central or median) and 590 i u per g (high) The average for the whole group was 290 i u

References p 372

This scheme has had some support from other investigators, but other methods are also used. Often only an average is quoted. We shall therefore usually include the average in presenting data, in spite of the drawback which has been already explained.

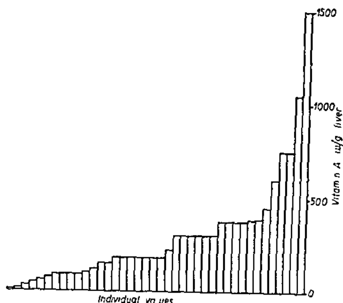


Fig 23 The vitamin A reserves of 40 inhabitants of various parts of Great Britain who died by accident during the years 1931–35 The individual values have been arrayed in ascending order Estimations by Lovibond Tintometer (Moore, 1937)

Liver reserves of vitamin A in various countries.

The first investigation on a large scale was made by Wolff [2, 3]. Similar studies followed in Britain [4–6], South Africa [7], China [8] and Norway [9]. The average and median values given in Table 40 usually refer to groups of adult subjects who had died by accident.

The differences between the results for different countries seem to be substantial, at least on paper. We must be cautious, however, before accepting them as real. Firstly we must remember that the calibration of instruments and methods 10–20 years ago was less dependable than it is now. Systematic errors between different laboratories cannot therefore be quite excluded. Secondly we have seen that the variations between individuals in any group are enormous In at least some of the investigations the medians might have been greatly modified if larger groups had been studied.

Perhaps the best evidence for the reality of the variation between countries may be found in the very low values reported for China by Woo and Chu Their median of 54 i u per g was only about one-sixth of the value of 324 i.u. found in Britain in the second survey by Moore [5]. It must be admitted, of course, that only 12 specimens were examined in the Chinese work. Medians

below the corresponding British levels however were al r
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TABLE 40

LIVER VITAMIN A RESERVES IN ACCIDENTAL DEATH

Country	Worker	Period	No of cases	Vitamin A i u /g	
				Average	Median
Holland	Wolff	1929 32	~8	160	110
Britain	Moore	1931–35	40	290	220
		1941 44	71	455	324
S Africa (natives)	Fox	1933	14	297	190
China	Woo & Chu	1939	12	70	54
Norway	With & Ødegaard	1943	12	166	153
Sweden	Dzialoszynski & Tomaszewski	1943 44	27	318	233
Scotland		1947	11	586	638

A suggestion that the vitamin A reserves in the same country may vary at different periods must arise from Moore s finding of a median of 324 i u per g for the war years of 1941 44 as compared with only 220 i u for the pre war period of 1931–35 Between these dates the fortification of margarine with vitamin A was made compulsory and a publicity campaign for an increased consumption of carrots had been conducted A rise in the liver reserves of vitamin therefore might well have been expected A difference between the two investigat that the d r
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a investigation 20 specimens came from Glasgow respective medians of 264 and 268 i u per g were virtually identical It remains possible therefore that the increase in the median for the whole country may have reflected differences in the place rather than in the time at which the specimens were collected

Vitamin A reserves in different age groups Wolff [2, 3] could find no clear evidence of any influence of age on the vitamin A reserves except for low values in infancy (Chap 21) In early work by Moore [1] and Ellison and Moore [10] lower reserves were found in old age and in childhood than in the group aged 15–59 years In later work the tendency of low values in old age was less pronounced while no further evidence was found for low values in childhood The median values found are given in Table 41 It is obvious that further investigation is needed

References p 37

before the influence of age on the vitamin A reserves is confirmed or denied

TABLE 41

VITAMIN A RESERVES FOUND IN ACCIDENTAL DEATH ARRANGED ACCORDING TO AGE GROUPS (MOORE *et al*, 1937 AND 1949)

Period	*Age group*	*No of subjects*	*Median vitamin A reserve, i u /g liver*
1931–35	15–60	40	220
	over 60	16	100
	4 mths –15 years	12	130
1941–44	15–60	71	324
	over 60	29	273
	4 mths –15 years	7	550

Magnitude of the total liver reserves of vitamin A

Taking 324 i u as the typical concentration of vitamin A in human liver, as found for the median value in Britain [5], and 1500 g as the weight of the liver we may calculate the typical total liver reserve of the healthy adult as roughly 500 000 i u

Carotenoids in liver

Carotenoids may be detected in the liver, but they do not follow vitamin A in being concentrated in this organ much above their level in other tissues Probably for this reason the hepatic carotenoids have attracted little attention

Vitamin A in the blood plasma

The literature on the levels of vitamin A and carotenoids in human blood [11–67] is much more extensive than that on the liver, presumably because specimens can be obtained more easily Four periods of progress can be recognised, which coincide with the sensitivity of the apparatus available In the first period the Lovibond Tintometer was used, mainly in Holland and the East Indies The concentration of vitamin A in the blood plasma is usually about 300 times less than in the liver, and the blue colours obtained with antimony trichloride from reasonably small specimens of plasma are very faint The tintometer was really unsuitable for taking readings at such intensities, and it is rather surprising that Wolff [17] reached conclusions which agree, at least at some points, with modern findings The second period may be linked with the Zeiss step-photometer, which was used in an important study by Lindqvist [27] In this delicate optical apparatus the solution under investigation is viewed through a filter which transmits light of a complementary colour, and the amount of absorption is measured by reducing the intensity of a parallel beam which does not pass through the solution A disadvantage was the length of time necessary to match the rapidly fading blue colour

The third era brings us to modern times It dates from Kimble's application to blood [28] of the photoelectric absorptiometer, which had previously been recommended by Dann and Evelyn [65] for the estimation of the vitamin in other sources. Rapid and accurate matching of the faint and transient blue colour was now possible, and a mass of data was accumulated by numerous investigators in many parts of the world. Finally in a fourth period of development the supremacy of the antimony trichloride procedure has been challenged by the neat micro-method of Bessey [52]. This method, which requires only a few drops of blood, depends on the measurement of the absorption at 328 mμ in an extract before and after the destruction of the vitamin by ultraviolet irradiation. (See Appendix)

A comprehensive review of all studies on the vitamin A contents of blood would present almost insuperable difficulties In the early Dutch work the results were usually expressed in Lovibond units, and their conversion in retrospect into international units is often a hazardous procedure. The use of alkali during the extraction procedure also introduces complications. If the digestion has been sufficiently prolonged the same results will be obtained as with Kimble's simple extraction with alcohol and ether, but if the time of digestion is short the results will often be low. Some workers make allowances for the contribution by carotenoids to the blue colour produced by antimony trichloride, but others neglect to make this correction. A further exasperating source of uncertainty arose through several investigators using the German vitamin A concentrate "Vogan" for the calibration

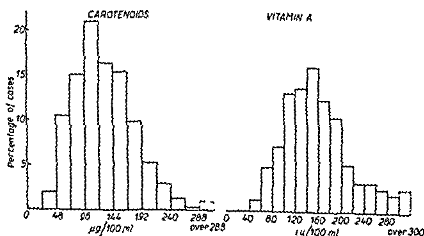


Fig 14 Distribution of values for vitamin A and carotenoids in the blood plasma of healthy human subjects according to arbitrary ranges The data were accumulated over the years 1952 and 1953 on specimens taken from 306 healthy inhabitants of Great Britain, including both sexes (Leitner, Moore and Sharman, unpublished data) Each column covers a range of 20 i u of vitamin A, or 24 μg of carotenoids The mode for vitamin A was between 140 and 160 i u per 100 ml, and for carotenoids between 96 and 120 μg.

References p 372

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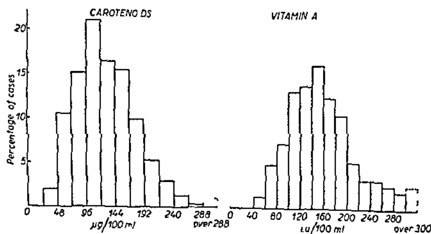


Fig 24 Distribution of values for vitamin A and carotenoids in the blood plasma of healthy human subjects according to arbitrary ranges The data were accumulated over the years 1952 and 1953 on specimens taken from 306 healthy inhabitants of Great Britain including both sexes (Leitner Moore and Sharman unpublished data) Each column covers a range of 20 i u of vitamin A or 24 μg of carotenoids The mode for vitamin A was between 140 and 160 i u per 100 ml and for carotenoids between 96 and 120 μg

References p 372

of their instruments A conversion factor of 3500 between ultraviolet readings and biological activity was claimed at one time for this preparation instead of the usual 1600 [69]. On this basis results for vitamin A would have been given more than twice their true value

As a further difficulty we have again, as with liver, to contend with series of results which vary widely between different individuals It is true that there appears to be a "mode" for vitamin A in blood (Fig 24), and that physiological evidence of deficiency appears when the vitamin falls below a certain level. But the main conclusions from the masses of data, so laboriously collected, must be drawn from comparisons between groups In Table 42 the results of several large scale modern investigations, mostly by Kimble's method, have been collected together

TABLE 42

VITAMIN A AND CAROTENOID CONTENTS OF THE BLOOD PLASMA OF HEALTHY ADULTS, IN GROUPS OF BOTH SEXES AND OF HIGH LIVING STANDARDS

Country	*Observers*	*No of subjects*	*Year*	*Total carotenoids µg/100 ml*	*Vitamin A i u /100 ml*
U S A	Kimble	64	1939	176	109
	Abels *et al*	124	1941	195	160
	Murrill *et al*	45	1941	206	104
	Popper & Steigmann	27	1943	81	153
	Harris *et al*	70	1946	210	201
Great Britain	Yudkin	23	1941	120	113
	Moore & Leitner	195	1944	92	121
	Campbell & Tonks	133	1947	80	108
	Leitner *et al*	219	1952	133	148
S Africa (Europeans)	*Highman*	*40*	*1944*	226	108
Norway	Ditlefsen & Stoa	100	1954	97	91
	Grand average	1040		137	131

It will be seen that the averages for carotenoids in different investigations ranged from 81 to 226 µg per 100 ml, and the averages for vitamin A from 91 to 201 i u Since all the specimens were collected from industrialised communities, with high standards of living, it seems reasonable to calculate the averages for the whole 1040 subjects The values obtained, without separation of the sexes, are as follows

Carotenoids 137 µg per 100 ml of plasma

Vitamin A 131 i u ,, ,, ,, ,, ,,

Discussion of the effect of sex, with male groups almost invariably ex-

ceeding the females in vitamin A but tending to be lower in carotenoids may be postponed until Chapter 35

Other lipoids in blood plasma

With the international unit of vitamin A weighing only 0 3 μg the foregoing averages imply on a weight basis that human plasma usually contains over three times more carotenoids than vitamin A Table 43 gives typical values collected from various sources for the main lipoid constituents of human plasma

TABLE 43

TYPICAL VALUES FOR THE LEVELS OF VARIOUS LIPOID COMPONENTS IN HUMAN BLOOD PLASMA

	μg per 100 ml
Total cholesterol	230 000
Neutral fat	*230 000*
Phosphatides	180 000
α Tocopherol	1 100
Carotenoids	140
Vitamin A	40
Vitamin D	Trace

Independence of liver and blood levels of vitamin A

We have seen in Chap 20 that the level of vitamin A in the blood is largely determined by factors other than the magnitude of the liver reserve It is not surprising therefore that Meyer *et al* [43] could find no correlation between the concentrations of vitamin A in liver specimens taken by biopsy and in blood from the same subjects

Although Gounelle *et al* [42] have reported considerable day to day variations in individual blood levels of vitamin A this experience has not been confirmed Most workers have found that in the absence of pyrexia dosing with vitamin A or other disturbing factors the level of vitamin A tends to remain at a constant level characteristic of the individual

Blood carotenoids in different countries and communities

We shall see later in this chapter that when volunteers are deprived of dietary sources of vitamin A and of carotenoids the level of carotene in the plasma falls rapidly On the other hand vitamin A declines only very slowly presumably because replenishments can be mobilised from the liver In line with these observations the plasma carotenoids are usually more responsive than vitamin A in providing an indication of the dietary habits of groups of people

Analysis of the data in Table 42 shows a wide difference between the levels of carotenoids between the U S A and Great Britain accompanied by a much smaller difference between the averages for Vitamin A Thus for 330

References p 372

American subjects carotenoids averaged 187 μg and vitamin A 150 i u per 100 ml For 570 British subjects the corresponding averages were 106 μg and 128 i u There seems no reason to doubt that the difference in carotenoids is genuine Probably the explanation lies in a greater use of vegetables particularly salad greens in America

Clear evidence of variations in blood carotenoids between different classes of subject were found in Britain by Leitner Moore and Sharman [64] Comparisons were first made between a large group of healthy private patients of Dr Leitner and a group of inmates of a mental hospital In each group both sexes were included The following result were obtained

μg or i u per 100 ml	Private patients			Mental patients		
	No of subjects	Range	Mean	No of subjects	Range	Mean
Carotenoids	220	15–371	133	179	18–137	53
Vitamin A	219	51–297	148	159	12 364	118

It will be seen that the average for carotenoids in the mental patients (53 μg) was less than half that found for the private patients (133 μg) Vitamin A was also lower in the mental patients but the divergence from the average for private patients was much less pronounced than for carotenoids In order to decide whether the differences were due to the diet or to the effects of the mental disease 50 patients were given 170 g daily of carrots or spinach as a supplement to their usual rations [67] The effect of these additions was as follows

μg or i u per 100 ml	Days of supplementation		
	0	17	44
Carotenoids	38	86	114
Vitamin A	125	156	155

The significance of the vegetable ration under practical conditions in influencing the blood carotenoid levels in a large group of subjects was therefore clearly demonstrated We shall see later that the differences in the blood provitamins before and after supplementation were even greater than would be suggested by the figures for total carotenoids This is because non carotene pigments make up a higher percentage of the total carotenoids when readings are low than when they are high

Levels of carotenoids which would be considered low in Britain or the U S A are commonplace in tropical countries Thus Lanzing [26] found an average of 48 μg per 100 ml for ten inhabitants of Batavia Burch *et al* [62]

collected blood from large numbers of Philipinos in Bataan who were mostly pregnant or lactating women During the year 1948 carotene averaged 47 μg per 100 ml and vitamin A 139 i u for 193 subjects while in 1950 the corresponding values were 72 μg and 112 i u for 187 subjects

The nature of the plasma carotenoids

Although the plasma pigments are sometimes described as carotene presumably for convenience it must be emphasised that several pigments are usually present and that in some circumstances the proportion of carotenes may be quite small Thus in 1937 Van Veen and Lanzing [22] found by chromatography that the blood carotenoids of Batavian subjects sometimes contained only about 15% of carotenes with cryptoxanthin xanthophyll and lycopene making more substantial contributions Kon and Mawson [70] detected β carotene lycopene lutein and an unknown pigment in the blood of British subjects Leitner Moore and Sharman [87] found that the percentage of carotenes was greater when total carotenoids were high than when they were low Their findings were as follows

Mean total carotenoids	*Percentage of carotenes*
102 μg per 100 ml	57
38	26

Magnitude of the total amounts of vitamin A and carotenoids in the blood plasma

Taking 131 i u of vitamin A and 137 μg of carotenoids per 100 ml as typical of the normal adult and 2 litres as the volume of the plasma we may calculate the total amounts circulating in the blood plasma at any time as 2620 i u and 2740 μg respectively As approximations 2500 i u of vitamin A and 2500 μg of carotenoids seem convenient round figures As stated in the preceding paragraph the contribution of carotenes to the total carotenoids is variable

The Vitamin A Status in Experimental Deficiency

We have seen that the average human subject at least in Britain has about 500 000 i u of vitamin A in his liver and about 2 500 i u in his blood plasma By forestalling matters discussed in our next chapter we may take the daily requirement as roughly 2500 i u On a purely mathematical basis therefore it appears that the liver reserves of vitamin A should suffice to prevent deficiency for 200 days The amount of vitamin A circulating in the blood plasma slightly exceeds the supply for one day

We have seen in Chapter 20 however that in experimental animals the liver reserves of vitamin A are sometimes used up more rapidly than would

References p 372

be expected from our knowledge of physiological requirements For this reason among others it is important to decide whether the large reserves found in the human liver do in fact give prolonged protection against dietary deficiency

Experiments to settle this point have included studies of the level of vitamin A and carotenoids in the blood plasma of volunteers restricted to a deficient diet In the absence of any readily apparent visible signs of deficiency however, some functional test of the adequacy of the vitamin A status is also necessary For this purpose measurements of the efficiency of dark adaptation have been extensively used

Dark adaptation tests A photometric test of the efficiency of dark adaptation intended for the detection of moderate degrees of dark adaptation was described in 1934 by Jeans and Zentmire [71] The subject first was required to look for a few minutes at a bright light, with the object of bleaching any visual purple in the retina The light was then turned off and in the darkened room the subject was asked to inform the observer as soon as he could perceive a pattern composed of faintly illuminated spots of light This time was recorded and the process repeated

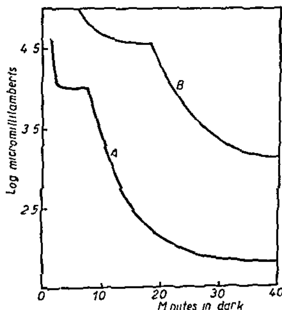


Fig 25 The dark adaptation curves of a volunteer in the Sheffield experiment (1942–44) recorded (A) at the commencement of the experiment and (B) after 17 months of subsistence on a diet deficient in vitamin A and carotene

with the pattern even more faintly illuminated By continuing the observations for about an hour a graph was obtained relating time with the millilamberts of illumination necessary for the perception of the pattern

Inspection of Fig 25 will show that the graph first follows a short curve,

which is associated with the activity of the retinal cones and then changes to a longer curve which is associated with the rods The effect of vitamin A deficiency is usually to increase the time necessary for the pattern to be seen at each stage of illumination There is also an increase in the final rod threshold which is the intensity of illumination necessary for the perception of the pattern even after prolonged adaptation The graph in deficiency therefore rises above the graph obtained when the subject is adequate in vitamin A

Several other forms of apparatus for measuring dark adaptation were devised[72, 77] In some the subject is asked whether he can perceive a flash of light rather than a constant source As another modification a fixation point of red light is sometimes included in the apparatus with the idea of ensuring that the flash should be focussed on a fixed and sensitive part of the retina With all forms of apparatus however certain precautions are necessary before faulty dark adaptation can be ascribed with confidence to dietary deficiency of vitamin A Thus poor dark adaptation may be associated with various diseases or with pregnancy The usual test to decide whether defective adaptation in any individual is related to vitamin A deficiency depends on finding whether heavy dosing with the vitamin causes improved adaptation

Early experiments on vitamin A deprivation

In 1939 Booher Callison and Hewston[78] reported the effects of restricting five adult volunteers to a mixed diet from which foods containing more than traces of vitamin A were omitted Dark adaptation was impaired after periods of 16 27 29 39 and 124 days but no keratinisation of the epithelial structures of the eye could be found on examination with a slit lamp (Chap 31) As they became abnormal in their dark adaptation the subjects were dosed with vitamin A for the purpose of determining the daily requirement which was necessary to prevent the deficiency from becoming more pronounced No chemical estimations were undertaken on the blood vitamin A or carotenoids In the same year Wald and Steven[79] described a similar experiment on a single volunteer Rather surprisingly the decision was made to dose the subject with vitamin A for 18 days before restriction to a deficient diet Nevertheless after 34 days a mild degree of impaired dark adaptation had developed When a dose of 20 000 i u of carotene was given a cure commenced after only 12 minutes and was complete within 90 minutes

More extensive experiments were next carried out in Germany by Wagner[80] Ten adult volunteers were given a diet deficient in vitamin A and its provitamins but adequate in other nutrients for 188 days Up to the 109th day the subjects gained in weight but subsequently their weights declined Dark adaptation became defective and there were changes in the degree of

References p 372

sensitivity of the retina to different colours In the blood the haemoglobin and erythrocyte count fell, and there was leucopenia with a "right shift" of the differential count (Chap 27). Cures could be affected by dosing with either carotene or the vitamin A concentrate Vogan

Further studies were made by Wald [81] on five young men Once again this worker loaded the scales against a successful demonstration of deficiency by giving his volunteers massive doses of the vitamin for a month before the main part of the experiment was started The most striking and rapid effect of deficiency was the virtual disappearance of carotenoids from the faeces A pronounced reduction in the blood carotenoids soon followed On the other hand the blood vitamin A retained its resting level as found at the beginning of the experiments Deterioration in dark adaptation could be detected in only two out of the five subjects There was no change in the capacity for physical work

Brenner and Roberts [82] dispensed with a preliminary dosing period advocated by Wald, but obtained substantially the same results For six volunteers the average for carotenoids fell from 228 μg per 100 ml before deprivation down to 39 mμ during deprivation, but vitamin A remained at about 20 i u per 100 ml before and after deprivation After periods of 4½ to 7½ months no evidence of impaired dark adaptation, nor any other signs of deficiency, could be observed in any of the volunteers

The 'Sheffield Experiment' in vitamin A deficiency We now come to what so far has been the most ambitious investigation on the effects of vitamin A deficiency in the human [83] It was conducted in Britain during the second world war, and its immediate object was to assist the Ministry of Food with information on vitamin requirements In particular data were required on the relative efficiencies of carotene and preformed vitamin A in satisfying these requirements, since the former could be produced at home while the latter had to be imported from America

Fortunately a group of some twenty volunteers, willing to act as experimental subjects, was available at Sheffield, having just completed an investigation on the propagation of scabies This team, which was supervised first by Dr Kenneth Mellanby and later by Professor H A Krebs was placed at the disposal of the Vitamin A Sub Committee of the Medical Research Council, with Miss E M Hume as Secretary A panel of about 30 medical and non-medical scientists was recruited with the intention of examining the effects of deficiency from all possible angles

Diet To give the investigation every hope of success great care was taken to exclude all sources of more than traces of vitamin A and provitamins from the diet A list of permitted foods known to contain only insignificant amounts of the vitamin was drawn up, and chemical tests

were made in any doubtful cases Special supplies of dried skimmed milk unvitaminised margarine meat bacon sugar and jam were made available by the Ministry of Food Foods eaten in large quantities such as bread and potatoes were examined for carotenoids in laboratories at Cambridge and Liverpool As an additional precaution specimen meals were collected dried and assayed for vitamin A by chemical means By this method both the Cambridge and Liverpool laboratories agreed that the average vitamin A intake of the volunteers not given supplements amounted to about 30 μg of carotene daily Finally specimens of the meals were collected minced up without drying and taken to Messrs Boots laboratories at Nottingham for testing upon rats The animals did better than others given a standard basal diet deficient in vitamin A in so far as they all survived over a period of 10 weeks They grew much less rapidly than animals given an adequate mixed diet however and had rough untidy coats At the end of 10 weeks they were found to have no reserves of vitamin A in their livers except for a trace in one animal

We may be confident therefore that the volunteers received a diet which was as low as it could be made in vitamin A activity without either resorting to a completely artificial diet or running risks of nutritional deficiencies other than of vitamin A Sixteen volunteers including two women received the diet at first without supplements for periods ranging from 11½ to 25 months Seven others were given the basal diet but with supplements of vitamin A or carotene from the start Most of the volunteers were conscientious objectors to military service and the mental attitude of self examination which they had shown in regard to the war made them meticulous in adhering to the restrictions which were imposed upon them

Effects of deprivation In the main group of sixteen subjects the only prompt effect of deprivation was a pronouced fall in the level of carotenoids in the blood plasma (Fig 26) Within the first two or three weeks of the experiment the average for the group fell from a preliminary value of 84 μg per 100 ml to only 12 18 μg Moreover it was found by chromatographic tests carried out at Reading that the residual yellow pigment responsible for the low readings was not carotene It appeared therefore that carotene had virtually disappeared from the plasma

In contrast the level of vitamin A in the plasma remained unchanged at least for the first two or three months The efficiency of dark adaptation measured by the final rod threshold was also unaffected The findings so far therefore confirmed those of Brenner and Roberts [82] and of Wald [81] in his second experiment Thus except for the immediate decline in carotenoids no evidence of the effects of deprivation was found either in chemical or in physiological tests

References p 372

With more prolonged deficiency, however, the observations on the group became less uniform and more hopeful that a significant decline in the



Fig 26 Fall in the average total carotenoid level in the blood plasma in 16 human volunteers who were given a diet deficient in vitamin A and carotene Breaks in the curve indicate the retirement of volunteers from the group The residual pigment which remained after the first few weeks was not carotene (Sheffield Experiment 1942–44)

vitamin A status might be achieved For the whole group the plasma vitamin A fell from an average of 88 i u per 100 ml at the start of the

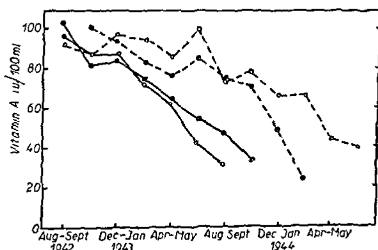


Fig 27 Levels of vitamin A in the blood plasma of the four volunteers in the Sheffield experiment (1942 44) who showed the most pronounced decreases The points represent two monthly averages

experiment to 72 i u about eight months later It was noticed, moreover, that the rate of decline in vitamin A differed considerably in different

individuals One volunteer, maintained a level equalling or exceeding his original value, which was somewhat above the average found for the whole group, for no less than 22 months In most of the other subjects a slow fall continued at about the rate at first observed for the whole group Four subjects could be picked out, however, in which the rate of decline was much more rapid (Fig 27) Eventually, after periods of 12–18 months, outstandingly low average values 24, 31, 34 and 40 i u per 100 ml were recorded for the last two monthly period in each case By the same time the dark adaptation had deteriorated significantly in the three of these subjects who had the lowest vitamin A levels In none of the other volunteers was dark adaptation defective except for a slight deterioration in winter which improved spontaneously in summer

Conclusions Our next chapter will include a review of the evidence on the daily requirement for vitamin A and carotene which was gained during the Sheffield experiment, mainly by studying the doses required for curing the three volunteers who developed defective dark adaptation For the present, however, it may be instructive to see how far the general findings at Sheffield can be correlated with those obtained in the studies on the vitamin A contents of human liver and blood, as described in the early parts of this chapter

We have seen that the typical liver reserve in Britain for a healthy adult at the time of the Sheffield Experiment was about 500,000 i u , which would be expected to suffice normal requirements for 200 days The results of the experiments show that this expectation has been realised, and even exceeded Thus we might expect the times causing depletion to be centred round 200 days, with some much shorter and others much longer In practice, however, the shortest times necessary for incomplete depletion was 400–500 days The observation that only four subjects could be depleted, while the rest could not, agrees with the widespread variation in vitamin A reserves found between different individuals

In contrast to the liver the blood contains an amount of vitamin A which would suffice the body's requirements for only two or three days Since carotenoids disappear rapidly from the blood during deprivation it seems probable that the vitamin A which is originally present in the blood is also fairly rapidly metabolised The conclusion seems unavoidable that as vitamin A is lost from the blood more is mobilised from the liver to take its place

It will be realised, of course, that the Sheffield Experiment, and the others which preceded it, suffered from two unavoidable handicaps In the first place the production of vitamin A deficiency in any animal population which is known to possess large and variable reserves of vitamin A will be a long and hazardous venture Thus no investigator would entertain for a

References p 372

moment the idea of using for that purpose a colony of adult well nourished rats, unless he desired to study the combined effects of deficiency and extreme old age Secondly the healthy adult, unless a pregnant or lactating woman (Chap 21) is subject to no special strain on the vitamin A metabolism, such as would be imposed by rapid growth or by illness The possession of a substantial reserve of vitamin is therefore unassailed by any specially urgent demand

In these circumstances it is not surprising that no clear evidence of deficiency was obtained in the majority of the volunteers who took part in the Sheffield Experiment The realisation of unquestionable signs of deficiency in three of the subjects represents a greater scientific achievement, and a greater tribute to the patience and reliability of the volunteers, than might at first sight be appreciated

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CHAPTER 30

The Dietetics of Vitamin A, including Requirements and Sources

Our knowledge of the dietetics of vitamin A is adequate in regard to the amounts of vitamin and provitamins that are present in various foods, but rather indefinite concerning the requirements necessary to maintain health The reasons for this disparity will be readily understood Chemical or biological examinations of foodstuffs can be carried out in the laboratory at any time, but opportunities for experiments on human requirements are not readily available Thus when frank cases of deficiency occur spontaneously in malnourished populations it is the clinician's duty to ensure prompt cures by massive dosing There must be no risk of the lesions becoming worse while the effect of more modest doses is first explored On the other hand when volunteers are prepared to submit themselves to experimental deprivation it is very difficult, and time-consuming, for a frank state of deficiency to be attained, as we have seen in the preceding chapter Even if a few volunteers can be made deficient, moreover, another difficulty must be faced in deciding on the criteria by which the adequacy of treatment can be assessed Is it sufficient to correct the functional abnormality of defective dark adaptation? Or should the allowance of vitamin also be capable of raising the level in the blood to the value found before deprivation was started?

The problem is further complicated of course, by the derivation of vitamin A activity either from the preformed vitamin or from its provitamins. Our first difficulty in this direction arises from the fact that the international units of vitamin A and carotene have been equated on the basis of experiments on rats, which appear to be more efficient than the human in utilising small doses of provitamins The value to the human of any source of carotene, even when difficulties of absorption are reduced by solution of the pigment in oil, tends therefore to be less than is indicated by the potency expressed in international units For this reason a daily dose of 1000 i u of vitamin A in oil will always be much more potent in the human than 1000 i u of carotene in the same medium Our second difficulty arises from the varying

References p 391

efficiency with which carotene is absorbed from different foodstuffs Thus the disparity between vitamin A and carotene in the human will be increased if the pigment is not given in oily solution, but as some partially indigestible vegetable

With so many points requiring further investigation it is not surprising that our assessments of human vitamin A requirements are still only rough approximations As our lower limit, which must be substantially surpassed if danger to health is to be avoided, we may take the vitamin A contents of diets which have been known to cause a high incidence of frank deficiency Thus observations in Denmark on the effect of a diet of skimmed milk, or in India on the effect of poor vegetable diets, will be relevant to fixing our lower limit As our upper limit we may take a diet known to be adequate for good health, with liberal amounts of milk, butter, eggs and green or yellow vegetables For guidance in deciding on a single figure between these limits typical of the average requirement, we must rely mainly on the scanty evidence obtained in work on experimental deficiency It may also be helpful, however, to review evidence on the vitamin A requirements of other species, which can usually be subjected to more drastic experimental conditions than are permissible in humans

Yet another group of problems must be faced when we attempt to go beyond a single figure for the vitamin A requirement as assessed for a healthy adult man or woman not subjected to the strain of reproduction and attempt to adjust our allowances for different types of individuals Again our information is very scanty We have clear evidence however, that lactation involves a high output of vitamin in the milk, and that certain diseases seriously affect the vitamin A status

In the face of all these perplexities it has been the duty of official committees to define allowances of vitamin A on which nutritional advice and policy can be based In this chapter we must first review and discuss such estimates, and next consider how they can be satisfied in terms of common foodstuffs, or of therapeutic preparations Attention will be drawn to the very wide variations in vitamin A and provitamin contents which are found between different foods, sometimes even when they are closely similar in type Consideration will be given to the effects of storage and cooking on vitamin A activity

Vitamin A Requirements of Humans

In discussing requirements we shall first consider the needs of a standard 70 kg adult, and later review such evidence as is available on special demands due to age, growth and reproduction

Diets adequate and inadequate in vitamin A

The classical examples of vitamin A deficiency in temperate zones have occurred in infants, or young children, who have been reared on a diet of farinaceous foods and potatoes, mixed with some form of skimmed cows' milk (Chap 31) From biological tests Booher and Marsh[1] have concluded that skimmed milk contains 17 i u per 100 ml The vitamin A contained in 8 6 litres which our adult must drink daily to satisfy an energy requirement of 3000 calories, therefore amounts to 1460 i u We may consider that this level is dangerously low, although in infants the intake may be further reduced by the inclusion of starchy foodstuffs which are negligible as sources of the vitamin

In India Aykroyd and Krishnan[2] observed signs of vitamin A deficiency, including Bitot's spots (Chap 31), in 27% of a large group of children, who subsisted on a poor vegetable diet The carotene intake for ages 1–12 years averaged 1140 i u daily which must be increased to 1700 i u for a standard adult Since the carotene was supplied in the form of vegetables we may conclude that the efficiency of absorption was low The Indian diet seems to have been even more inadequate than a regime of skimmed milk in supplying vitamin A

As typical of a diet adequate in vitamin A we may take whole milk as our example We have seen in Chapter 21 that during full lactation the milk of most species contains about 140 i u of vitamin A sometimes with the addition of smaller amounts of provitamins To meet his energy requirements the adult would require 4 4 litres daily which would provide about 6200 i u of vitamin A

Experiments by Lewis and Barenberg[3] however have suggested that the vitamin A provided by a diet consisting mainly of whole milk greatly exceeds the minimum requirement Thus when large groups of children were kept for several months on a diet containing only one quarter of the normal amount of vitamin A they showed no inferiority either in growth or in resistance to infections when compared with normally nourished infants On this evidence the requirement for the standard adult would be reduced to about 1500 i u

The scanty evidence available on these lines may be summarised as follows

Reference	*Food*	*Daily intake of vitamin A or carotene i u*	
		Found adequate	*Inadequate*
Bloch (Chap 31)	Skimmed milk		1460 (vit A)
Aykroyd & Krishnan	Veg diet		1700 (cart)
General experience	Whole milk	6200 (vit A)	
Lewis *et al*	Part skimmed milk	1500 (vit A)	

References p 391

Early estimates of vitamin A requirements

Official estimates of the intakes of vitamin A necessary to maintain health have suffered from the weakness of available evidence both on the minimum dose necessary to prevent acute deficiency and on the increase above the minimum dose which should be allowed as a safety margin It has perhaps been a convenient avenue of escape to harassed committees that the different values of the international units of vitamin A and carotene arising from the inferior use of carotene by the human as compared with the rat have allowed considerable ambiguity in the interpretation of a single value chosen as the official requirement

In 1938 the Technical Commission on Nutrition of the League of Nations [4] approved 2000–4000 i u daily as a safe allowance of vitamin A It was recommended that this allowance could be supplied by 500 ml of milk 1 egg 25 g of butter and a medium sized helping of a green leafy vegetable These foods would supply nearly 3000 i u as preformed vitamin A alone and should protect members of well fed communities against any danger of deficiency In populations subsisting predominantly on vegetable foods however the intake of preformed vitamin A may be very low and more reliance must be placed on provitamins The League of Nations Commission made no suggestions as to how requirements can be satisfied when liberal amounts of dairy products are not available

In 1943 the Food and Nutrition Board of the U S A National Research Council [5] quoted the Recommended Allowance of vitamin A for a 70 kg or 56 kg woman as 5000 i u It was suggested that the requirement might be less if provided as preformed vitamin A and greater if provided as carotene Without detailed advice as to how these adjustments should be made however the Board s recommendation seems too ill defined to have much practical value

Curative dosing with vitamin A or carotene

Various studies on experimental vitamin A deficiency have been mentioned in the preceding chapter We must now turn our attention to the attempts made in the course of these studies to find the minimum doses of vitamin A and carotene which were required to correct the effects of deficiency

Booher Callison and Hewston [6] observed defective dark adaptation in five volunteers after periods of deprivation of 16–124 days Normal adaptation could be restored by cod liver oil in doses of 1550–3850 i u per 70 kg For carotene the requirements were 3010–7210 i u We may note however that the periods of deficiency which caused defective adaptation in these experiments were much shorter than those found necessary by most other investigators

Wagner [7] found that dark adaptation was defective in ten adult male

volunteers after they had been deprived of vitamin A for about 6 months. In five subjects, who were dosed with the vitamin A concentrate "Vogan", doses of 2500 i u , but not 2000 i u , were stated to restore normal adaptation. As mentioned in Chapter 29, however, it is possible that a fault in calibration occurred, owing to the potency of Vogan being assessed at more than twice its true value After correction for this anomaly the curative dose of vitamin A appears to be only 1140 i u In five other subjects, who were treated with carotene, doses of 5000 i u were found necessary to restore normality.

From the preceding chapter it will be remembered that in the British Experiment at Sheffield[8] dark adaptation became defective in only three out of 16 subjects who were kept for long periods on a deficient diet The first of the depleted subjects was dosed, after restriction for 14 months to the deficient diet with 1300 i u of preformed vitamin A daily The vitamin was administered in the form of distilled natural esters dissolved in arachis oil Improvement in dark adaptation was noticed after dosing for two weeks, and steady improvement occurred for six months The level of vitamin A in the blood plasma, which before dosing was only 22 i u per 100 ml, rose after dosing to 50–88 i u Increasing the dose to 2600 i u had little effect on either dark adaptation or on the level of vitamin in the blood Restoration of a normal diet increased the blood vitamin A to within the normal range, but had no additional effect on dark adaptation

The second depleted subject was dosed, after deprivation for 18 months, with a solution of carotene in arachis oil After daily doses of 1250 i u had been given for a month the level of vitamin A in the blood had increased from 28 to 63 i u , but dark adaptation had further deteriorated The dose was therefore increased to 2500 i u Continuation of this treatment for nearly 6 months caused slow improvements in both the blood vitamin A and dark adaptation, but dark adaptation did not attain the efficiency subsequently observed when a normal diet had been restored Dosing with carotene increased the level of carotenoids in the blood Thus the levels before dosing, after one month's dosing with 1250 i u of carotene, and after 6 months dosing with 2500 i u were 12, 24 and 42 μg respectively

The third depleted subject was started with a dose of 2500 i u of carotene after restriction for 22 months The response was dramatic, with an improvement in dark adaptation within 7½ hours The responses during 3 weeks after dosing were as follows

	Final rod threshold	*Plasma/100 ml*	
	log µmlamberts	*Vit A, i u*	*Carts µg*
Before dosing	2 28	19	17
2500 i u cart for 7 days	1 68	59	16
2500 i u cart for 21 days	1 67	96	31

References p 391

No further improvement in dark adaptation resulted from restoration to a normal diet It is interesting that an increase in the blood vitamin A occurred after the first week of dosing without any corresponding rise in the carotenoids, and that at this point the maximum efficiency in dark adaption had already been attained

Comparison of human requirements with those of other species

An attempt to deduce human requirements from those found for other species was made by Guilbert, Howell and Hart[9] In extensive studies on cattle, sheep, pigs, horses and rats closely similar minimum requirements were found (Chap 34), which may be averaged as 18 3 i u of preformed vitamin per kg or 47 i u of carotene For the human a round figure of 20 i u per kg was taken for the minimum requirement of vitamin A and 40 i u for carotene making the requirements of the standard adult 1400 i u and 2800 i u respectively The intakes necessary for ' significant" storage of vitamin A in the liver, optimum dark adaptation and reproduction were assessed as three times greater at 4200 i u for vitamin A, and five times greater at 14 000 i u for carotene

The estimates of minimum requirements made for a 70 kg adult may now be summarised as follows

Worker	*Minimum dose (i u) of* *Vitamin A*	*Carotene*
Booher Callison and Hewston	1550–3850	3010–7120
Wagner	2500 (1250 ?)	5000
Medical Research Council	1300	2500
Guilbert Howell and Hart	1400	2800

In passing we may recall from Chapter 20 the author s view that the vitamin A requirements of different species can be more fairly compared on a basis of food intakes than on body weights The conclusions of Guilbert and his colleagues might appear to contradict this theory, since the require ments of the rat are considered to be in line with those of other species on a basis of body weight We may note, however, that although an intake of 60 i u per kg would cause ample storage of vitamin A in human liver the same intake would allow only very slight storage in the rat For the com parison between the human and farm animals the disparities in size are probably not great enough to reveal any anomaly when intakes are compared on a weight basis

Special demands for vitamin A

There is clear evidence that many factors must affect the requirement for vitamin A Thus up to 1000 i u of preformed vitamin A daily may be secreted during lactation During pregnancy there is a smaller demand, to provide vitamin A

for the liver of the foetus If we take the weight of the foetal liver as 100 g with 50 i u of vitamin A per g the amount actually transferred is about 5000 i u The quantitative demands of factors other than those concerned with the female side of reproduction however are much harder to assess We shall see in Chapter 32 that in pneumonia 1000 i u of vitamin A daily may sometimes be lost in the urine Smaller amounts may be lost over long periods in certain chronic diseases

Apart from these visible strains on the vitamin A status however other invisible demands must be presumed Thus the vitamin may be used up during rapid growth or in maintaining the deteriorating body fabric during old age In many illnesses the vitamin contents of the blood and liver may be reduced either without loss in the urine or with a loss which accounts for only a small fraction of the missing vitamin An increased rate of metabolism and presumably an increased demand must therefore be presumed The influence of hormones particularly adrenal thyroid and sex must not be overlooked In some diseases poor absorption of either vitamin A or carotene may necessitate increased intakes

With the knowledge at present available it will obviously be impossible to suggest intakes corresponding with every form of stress on vitamin A metabolism It seems reasonable however to make generous provision of vitamin A during rapid growth and reproduction and in old age Patients recovering from diseases known to affect the vitamin A status should be given massive doses to make good their losses

Conclusions on human vitamin A requirements

Having reviewed most of the scanty evidence which is available we must now try to condense this evidence so as to derive quantitative assessments of requirements which can be put to practical use We must also consider the calculations particularly in equating units of vitamin A and of provitamins which are necessary to avoid erroneous conclusions

The few curative tests possible in the investigation organised by the Medical Research Council have given results in good agreement with the experience of other workers As round figures it seems reasonable to take 1250 i u of vitamin A and 2500 i u of carotene both in oily solution as minimum requirements We must remember however that these doses gave plasma levels of vitamin A and carotene which were much below those of normally nourished subjects The consumption of an ordinary diet in a temperate country therefore allows a safety margin above the minimum requirement If a margin of 100% is allowed for safety as advocated by the Medical Research Council our recommended allowances of vitamin A and carotene in oil become 2500 and 5000 i u respectively

We must now face the complication that carotene is consumed in greater

References p 391

amounts in the form of vegetables than in oily solution Allowance must therefore be made for the less efficient absorption from vegetable foods which will vary according to the source concerned If we refer back to Chapter 16 where data are given on the maximum percentages of carotene absorbed from various sources we may calculate the amounts of typical vegetable food necessary to satisfy our requirements as follows

	Minimum dose	*Safety dose*
β Carotene in margarine	2600 i u	5200 i u
Spinach canned purée	4500	9000
Spinach canned homogenised	4300	8600
Cabbage dried outer leaves	4500 7000	9000–14000
Carrots canned homogenised	3300	6600
Carrots canned sliced	7700	15400
Carrots canned sliced puréed	7400	14800

It must be emphasized however that the percentages of absorption from the various sources have been calculated as the difference between the amount of carotene ingested and the amount excreted in the faeces The procedure assumes that the amount of carotene lost by destruction in the intestines is the same for each source and is so small that it can reasonably be neglected Obviously if carotene in oily solution were proved to be less stable than in vegetable sources during passage through intestines it would be necessary to reduce the estimated requirements of vegetables

The B M A scales Many gaps in our knowledge of vitamin A requirements still remain and it seems doubtful whether completely satisfactory information will ever become available For assessing the adequacy of diets in vitamin A particularly for official purposes it is therefore necessary to accept scales of allowances which are both consistent with the existing evidence and simple enough for routine application by working dieticians On these grounds the scales proposed by the Nutrition Committee of the British Medical Association [10] seem to the author to be worthy of general acceptance From Table 44 it will be seen that the recommendations are based on an allowance of 2500 i u of vitamin A as suggested by the Medical Research Council Carotene irrespective of its source is counted as three times less potent than vitamin A The special requirements in childhood pregnancy and lactation are taken into account No special provision is made for old age but the desirability of such provision must for the present be inferred from analogy with animal experiments rather than from direct experiments on humans

TABLE 44

DAILY REQUIREMENTS OF VITAMIN A FOR HUMANS AS ASSESSED BY THE BRITISH MEDICAL ASSOCIATION (1949 50)

hildren (15 years and under)		Vitamin A	1500 i u	or
		Carotene	4500 i u	or
	mixed diet	1/3 Vitamin A		
		2/3 Carotene	3000 i u	
ddolescents and adults		Vitamin A	2500 i u	or
		Carotene	7500 i u	or
	mixed diet	1/3 Vitamin A		
		2/3 Carotene	5000 i u	
Pregnancy		Vitamin A	3000 i u	or
		Carotene	9000 i u	or
	mixed diet	1/3 Vitamin A		
		2/3 Carotene	6000 i u	
actation		Vitamin A	4000 i u	or
		Carotene	12000 i u	or
	mixed diet	1/3 Vitamin A		
		2/3 Carotene	8000 i u	

Sub standard intakes of vitamin A

Finally a warning seems necessary against statements on the occurrence of vitamin A deficiency in populations when the evidence is based merely on the results of dietary surveys If accurate surveys indicate that the intake of vitamin A and carotene falls below the accepted allowance it is of course perfectly correct to describe the diet as sub standard The description of a population as deficient in vitamin A however should only be applied when dark adaptation is subnormal or some other pathological sign of avitaminosis can be detected in a significant proportion of the subjects examined Some reports of deficiency it may be suspected have arisen from nothing more than a high estimate of the requirement combined with the use of tables in which low potencies have been ascribed to certain important foods

Dietary Sources of Vitamin A and Carotene

If we are prepared to accept the vitamin A allowances proposed by the British Medical Association as offering the best alternative to precise and reliable estimates authenticated by an international body we may next proceed to calculate how these allowances can be supplied in the diet A really comprehensive statement of all the evidence available on the vitamin A and carotene contents of foods all over the world with a full account of all the factors affecting the vitamin contents of individual foods could in itself fill many chapters Pressure of space therefore necessitates that data should be given only for a selection of the more important foodstuffs

References p 391

As our main sources of information we may use the official food tables compiled in 1945 by the British Medical Research Council[11] and by the Bureau of Human Nutrition and Home Economics of the U.S. Department of Agriculture[12]. Several of the values given for carotene in the Medical Research Council's tables were based on estimations carried out by the author's colleague, Dr Vernon Booth. This worker has since made extensive

TABLE 45

COMMON DIETARY SOURCES OF PREFORMED VITAMIN A

Authority	*Vitamin A*	*i u /100 g or ml edible portion*		
	M R C (11)	*U S D A* (12)	*Booher* (1)	*Rounded figure**
Dairy products				
Milk, whole, fresh, summer	140		230	150
whole, fresh, winter	70	160	87	75
skimmed, fresh	0	Tr	17	17
whole, dried	1070	1400	1600	1100
skimmed, dried	30	40	140	70
whole, condensed, unsweetened	370	400		400
whole, condensed, sweetened	370	430	280	400
Cheese, whole-fat types	1300	1740	1200	1500
cottage (low fat)		30		30
Butter	4000	3300	5900 S 3650 W	3500
Ice cream		540**		500
Eggs				
Hens' eggs, whole, fresh	1000	1140		1100
whole, dried	3000	4460		4000
yolk, fresh		3210	3760 S 1880 W	3500
Liver and offal				
Liver, calf	4000			4000
ox	15 000			15 000
pig	5000			5000
sheep	45,000			45,000
chicken cooked			24,000	25 000
Kidney, ox	1000		1100	1000
Heart, ox	200	0	200	200
Meats and meat fats				
Beef and veal	50	0	0	20
Mutton and lamb	50	0	0	20
Pork and bacon	0	0	0	0
Fish				
Herring, fresh	94			100
Salmon canned	250	80		250
Sardines, canned	270	290***	136	250
Tuna canned		70***	200	100
Margarine (vitaminised)	2000			2000

* Including allowances for carotene

** Calculated from ingredients

*** These values relate to the drained contents of the can According to U S D A the oil surrounding the fish is much richer in vitamin A than the fish itself

and highly accurate estimations on many specimens each of a large number of sources and a table covering his findings is included with his kind permission in the appendix When it supplies the necessary information this table has been preferred over the older M R C table in drawing up the composite tables which are included in this chapter Help has also been obtained from a paper in 1941 by Booher and Marsh [1] which describes the estimation of vitamin A in 128 foods by means of biological tests From the preceding section the difficulties involved in the essential step of ex-

TABLE 46

THE CONTENTS OF VARIOUS FOODSTUFFS IN CAROTENE AND OTHER PROVITAMINS

	i u per 100 g			
Authority	*M R C (11) or Booth (chem)*	*U S D A (12)*	*Booher (1) (biol)*	*Rounded figure*
• Carrots mature	20 000	12 000	10 000	20 000
young	10 000			10 000
• Mint	19 000			19 000
Parsley	14 000			14 000
• Spinach	13 000	9 420	4 100	13 000
Dandelion leaves		13 650	9 000[a]	13 000
Spinach beet leaves	11 000	6 700	16 100[a]	11 000
Turnip leaves	10 000	9 540	15 700	10 000
Cress	8 300			8 000
Kale	8 000	7 540	10 500	8 000
Collards		6 870	8 800	8 000
Mustard greens		6 460	10 200	8 000
• Sweet potato		7 700	3 800	6 000
Watercress	5 200			5 000
Endive			3 850	4 000
Broccoli		3 500	3 500[b]	3 500
• Apricots	690	2 790	5 800[c]	2 000
Lettuce	2 600	1 620	210	2 000
• Tomato	1 300	1 100	1 300	1 200
Asparagus		1 000	960[a]	1 000
Bean French	950			1 000
• Cabbage	*900*	*80*	*100*	*500*[d]
• Peach	653	880	1 670	800
Brussels sprouts	700	400	640[e]	700
Bean runner	650			650
Water melon		590	500	550
Banana		430	400	400
Yellow maize		390	333	350
Gooseberry	230		380	300
Orange juice		190	250	200

(a) Cooked
(b) This value is for flowers the leaves contained 7300 i u per 100 g
(c) Dried
(d) The value is greatly affected by sampling according to whether the outer green or inner white leaves are taken
(e) Frozen and cooked

References p 391

pressing carotene values in terms of vitamin A will already have been realised It will be convenient, therefore, to make separate tables for foods of animal origin, which supply preformed vitamin A (Table 45), and of vegetable origin, which supply provitamins (Table 46)

Fish-liver oils have been excluded from Table 45 since they may perhaps be regarded as medicines rather than as foods The dietitian, however, is doubtless familiar with cod liver oil, for which a value of 1000 i u per g may be taken as typical of a good medicinal quality Halibut liver oil is also well known, with a typical vitamin A concentration of 50 000 i u per g Many other fish-liver oils have also been used as sources of vitamin A, but only a few have been marketed under the name of the individual fish For convenience fish oils listed in separate tables 64-72 in the appendix

The richest dietary source of carotene is red-palm oil, which is used as a cooking fat in parts of Africa Calculations from the data of Hunter and Krakenberger[13] indicate a typical provitamin concentration of 1000 i u per g The oil is omitted from Table 46 since no data are available from the three authorities who are quoted Details for various types of oil are given in the appendix

Even with the division of data between separate tables for vitamin A and carotene several complicating factors remain In some foods of animal origin preformed vitamin A is accompanied by carotene In milk and butter, particularly from cows of the Channel Island breeds, carotene may make a significant contribution to the total vitamin A activity of the food In most other animal foods, however, the contribution by provitamins is usually small The values given in Table 45 may be taken as including provitamins when they are present in significant amounts On the other hand very small contributions by provitamins, as in liver, have been ignored

In regard to Table 46 a more serious problem arises, since some of the data have been obtained by chemical tests and others by biological tests From Chapter 16 we may recall that the rat, in common with the human, appears to absorb carotene less readily from vegetables than from an oily solution, such as is used to distribute the international standard carotene. In biological tests with rats, therefore, vegetables will tend to give values lower than the chemical estimations might lead us to expect The ratio between the chemical and biological values will vary with the nature of the vegetable, but as a general rule we might divide our chemical value by a factor of 2 in order to predict the value assayed in rat tests In Table 46 a factor might be expected to operate between the values found by the Medical Research Council (column 1) and by Booher and Marsh (column 3) It is perhaps indicative of the difficulty of the problem that no consistent divergence can be found between these two sets of results

To assess the value of the vegetable for humans, moreover, another step is necessary. Earlier in this chapter we have mentioned that carotene, even in oily solution, is less efficiently absorbed by the human than by the rat. A further division of the chemical value, is therefore necessary, and again a factor of about 2 seems appropriate

In preparing the table, therefore, a choice had to be taken between giving precedence to the "gross" carotene values, as found in chemical tests, and the "net" values for humans which would take into account the points raised in the two preceding paragraphs On the whole it seemed preferable, and less liable to misunderstanding, to give precedence to the "gross" values When in human dietetics we desire to equate these values with international units of preformed vitamin we can divide so as to allow for the "rat–human" and "oil-vegetable" ratios For this purpose a combined factor of 3 has gained some measure of official acceptance [11] Alternatively factors could be chosen in accordance with the efficiency of absorption of carotene from the particular vegetable in question To equate carotene values obtained by rat tests with their equivalent of international units of preformed vitamin A in the human we must presumably divide by a factor of 2

In both Tables 45 and 46 the author has reviewed the data available for each food, and has assessed a "rounded figure" which in his opinion is the best approximation based on such evidence as is available Space precludes a detailed and critical review of all the evidence for every food

Variations in the vitamin A potency of individual foods

It must be emphasized that the values given in Tables 45 and 46 should be taken as typical for the foodstuff concerned, and not as being applicable to every individual specimen of that foodstuff Some variation is found for every food, but the ranges are much wider in some instances than in others Thus milk taken from cows at pasture will vary in its vitamin A potency according to the stage of lactation, but the combined milk of herds will usually provide about 150 i u per 100 ml In contrast liver is much more variable in its potency According to Moore and Payne [14] calf livers contained 0 6–225 i u per g, ox livers 6–450 i u, sheep livers 300–825 i u, and pig livers 0–150 i u The carrot is another food which shows a wide range of variation, with its carotene contents influenced both by variety and the degree of maturity [15] By special breeding varieties containing more than twice the usual level of provitamin may be obtained Mature carrots always contain more carotene than specimens of the same variety harvested while still immature

Variations between different foods

Apart from the question of variations in potency for individual foods we may see from Tables 45 and 46 that the average potencies of different foods are

References p 391

spread over very wide ranges Liver and liver oils provide by far our most concentrated sources of preformed vitamin A Even in this class however values for fresh liver range from an average of 63 i u per g for calf liver up to 468 i u for sheep Marine liver oils can differ by at least a thousandfold in their vitamin A activity Eggs dairy products and kidney are our next richest sources and are accompanied by margarine which has been fortified with vitamins Next we must mention fat fish such as salmon and herring and also fish roes Small traces of vitamin are contributed by beef and possibly by mutton There remain many fish and meat products which probably carry small amounts of vitamin A These traces might be enough to make the foods in question undesirable as components of an experimental diet deficient in vitamin but would be too small to prevent at least partial deficiency if the other dietary components were devoid of the vitamin

Provitamin sources also show wide differences Red palm oil and carrots are the richest readily available sources in which the yellow colour is not masked by chlorophyll Other yellow or red sources are the sweet potato tomato apricot and yellow maize Among green vegetables spinach turnip tops parsley watercress all approach the carrot in potency Cabbage and lettuce although not to be ignored are much less potent Orange juice contains small amounts of provitamins

Foods which are virtually devoid of either preformed vitamin A or carotene are listed in Table 47 It will be seen that many important staple foods are included Thus the cereals wheat rye barley and rice are generally considered to contain only traces of provitamins Extracts from wheat are certainly yellow but the pigment is predominantly xanthophyll Other major foodstuffs in the list are pork bacon lard vegetable cooking fats and many foods used for spreading on bread such as syrup honey and most kinds of jam With so many foods deficient in the vitamin it is perhaps difficult to understand why frank vitamin A deficiency is seen comparatively rarely Presumably the efficiency of storage of the vitamin allows a period of temporary plenty to provide protection over long periods of deficiency

Dietary ratios between vitamin A and its provitamins

Interesting questions arise over the proportions of preformed vitamin A and provitamins which should be consumed in making up the allowances which are considered necessary to ensure health An inhabitant of Europe or America can easily exceed 2500 i u of preformed vitamin A per day by eating 25 g of butter 500 ml of milk and an egg [4] Vegetables remain a desirable constituent of the diet but are not essential for making up the vitamin A allowance Inhabitants of tropical countries however may receive very little preformed vitamin A or even none at all Almost complete dependence therefore must be placed on provitamins

TABLE 47

FOODS DEVOID OF MORE THAN TRACES OF VITAMIN A AND ITS PROVITAMINS

Cereals	*Other vegetables*	*Fats*
Wheat[a]	Cauliflower	Lard
Oats	Cucumber	Palm kernel oil
Barley	Onions	Arachis (peanut) oil
Rye	Celery	Olive oil
White maize		Cottonseed oil
Rice	*Nuts*	Other vegetable oils
Sago	Almond	Margarine[b]
Tapioca	Barcelona nut	
Macaroni	Brazil nut	
Spaghetti	Chestnut	
Noodles	Cobnut	*Carbohydrate*
Biscuits	Coconut	Sugar
	Peanut	Syrup
Roots and tubers	Walnut	Treacle
Potatoes		Honey
Turnips		Most forms of jam
Swedes	*Meat and meat products*	
Beet	Pork	
Radishes	Bacon	*Miscellaneous*
Parsnips	Ham	Yeast
		Marmite
Pulses		Alcoholic drinks
Haricot bean	*Fish*	Meat extracts
Soya bean flour	White fish	Egg white

(a) In the author s experience a d et containing substantial amounts of wheat germ provides enough carotene to allow the survival of rats but not enough for the storage of vitamin A

(b) Unfortified

It is instructive to calculate the amounts of various foods which would be necessary to provide the equivalent of 2500 i u of preformed vitamin A on the assumption that the diet contained no other source of vitamin This calculation is made for a few typical foods in Table 48 It will be seen that the amounts required vary from 50 mg of halibut liver oil up to 15 litres of skimmed milk

The effect of cooking and food processing on vitamin A

The losses of either vitamin A or provitamins during domestic cooking are so small as to be negligible The losses which occur as the result of canning are also usually very small In foods preserved by dehydra tion however loss of either vitamin A or carotene can readily occur during subsequent storage Thus in dehydrated carrots the carotene sometimes tends to deteriorate giving a smell of β ionone [16] Vitamin A in the author s experience is sometimes lost on storage from liver which has been freeze dried No doubt such losses can be prevented or at least reduced by the storage of the dehydrated product under anaerobic conditions

References p 391

TABLE 48

DAILY INTAKES OF VARIOUS FOODS NECESSARY TO SATISFY A REQUIREMENT OF 2500 I U OF VITAMIN A OR 7500 I U OF CAROTENE[a]

Food	*Intake*	*Food*	*Intake*
Halibut liver oil	50 mg	Hen's egg	227 g
Cod liver oil	2 5 g	Ox kidney	250 g
Red-palm oil[b]	5 0 g	Apricot	375 g
Sheep liver	5 5 g	Lettuce	375 g
Ox liver	16 5 g	Tomato	625 g
Carrot mature	37 5 g	Canned salmon	1 kg
Calf liver	62 5 g	Cabbage	1 5 kg
Spinach beet leaves	68 g	Milk summer	1 67 l
Butter	71 5 g	Yellow maize	2 14 kg
Sweet potato	125 g	Herrings, fresh	2 5 kg
Margarine	125 g	Beef or mutton	6 25 kg
Cheese (whole fat)	167 g	Skimmed milk	15 l

(a) The amounts are calculated on the assumption that the food in question is the only source of vitamin A in the diet

(b) The requirement is taken as 5000 i u, because the carotene is in oily solution

The stability of vitamin A in pharmaceutical preparations Factors which cause the destruction of vitamin A have been mentioned in Chapter 9 Dietitians will hardly need to be reminded that vitamin A is unstable if it is exposed to air in oily solution, and that the rate of loss is increased if the vitamin is exposed on the surface of a solid, such as a proprietary food Fortunately warning of the impending destruction of the vitamin is usually given by an objectionable taste or odour, at least in oily solutions of the vitamin The action of air is accelerated by heat and light Smith [17] found that the rate of loss of vitamin A on storage in various oils was roughly proportional to the rate of formation of peroxides Rao [18] agreed that peroxide formation was an important factor, but concluded that the two processes were not coincident Thus peroxides were formed first, and were themselves broken down during the destruction of the vitamin

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CHAPTER 31

Clinical Observations on Vitamin A Deficiency

Allusions to night blindness by Egyptian and Greek writers in the pre Christian era have been already mentioned in Chapter 1 Apart from such isolated accounts, however, the growth of modern systematic knowledge of clinical vitamin A deficiency appears to date from about the middle of the 19th century In a review published in 1923 Blegvad[1] drew attention to a reference to keratomalacia by Arlt as early as 1851[2] The condition was observed in a young boy with scarlet fever and also in a young infant, both of whom also suffered from diarrhoea Other early reports of eye symptoms now associated with vitamin A deficiency came from Hubbenet[3], Bitot[4], Netter[5], Blessig[6], Gama Lobo[7] and v Graefe[8] These early observations, which were made in countries as widely apart as France, Russia and Brazil established that the functional defect of hemeralopia was often accompanied by injuries to the structure of the eye, which included xerophthalmia and lesser lesions Thus in a foundling hospital at Bordeaux it was noticed by Bitot in 1863 that defective dark adaptation was accompanied by characteristic spots on the conjunctiva, which have since borne his name

The point at which a dietary defect was generally accepted as the prime cause of the ocular lesions is now difficult to discern We may remember from Chapter 22, however, that in 1876 Simeon Snell, of Sheffield, was called upon to treat children who were suffering from night blindness accompanied by Bitot's spots They responded promptly to his prescription of cod liver oil and iron[9] The passage of time also makes it difficult to trace the first evidence relating ocular abnormalities with hyperkeratosis of the skin, but de Gouvea in 1883[10] and Mori in 1904[11] may be mentioned as early workers in this field Subsequent experience has indicated, of course, that skin abnormalities rather similar to those seen in vitamin A deficiency can also result from other causes

In this chapter we shall first review descriptions of the pathological effects of vitamin A deficiency, as seen in the form of ocular lesions, in various parts of the world We shall next summarise the evidence which

indicates that vitamin A plays a role in the prevention of skin lesions. Attention will be drawn to the effect of various diseases in precipitating vitamin A deficiency. The reverse danger, that vitamin A deficiency may precipitate various diseases, will be discussed in later chapters

Ocular Lesions in Acute Vitamin A Deficiency

Xerophthalmia in Denmark Bloch's classical contributions [12–14] to our knowledge of the effects of vitamin A deficiency were made possible by two circumstances Firstly economic conditions in Denmark before and during the 1st World War led to the excessive export of the dairy products for which this country is famous, with the result that babies in the poorer classes were often reared on sweetened skimmed milk In the light of later knowledge, therefore, it is clear that a high incidence of vitamin A deficiency was inevitable Secondly the clinical observations were made soon after medical interest throughout the world had been aroused by the discovery in America of the existence of vitamin A, and of the experimental production of xerophthalmia in rats given diets devoid of the vitamin

Bloch emphasized that xerophthalmia did not appear as a first symptom in an otherwise healthy child but usually came after the general condition of the child had been weakened by illness The first ocular abnormality was hemeralopia but this disability was difficult to detect in young infants. Xerosis or drying, of the conjunctiva was the first visible stage Later this xerosis extended over the cornea which first shrivelled up, and then developed regions of necrosis with ulceration and often perforation The stage known as 'keratomalacia' had then been reached In most cases the picture was complicated by infections The child so afflicted had photophobia, the conjunctiva were red and swollen, and the eyes began to water In keratomalacia the infection and inflammation spread to the interior of the eye, and sometimes resulted in extrusion of the lens Ultimately the original xerotic lesions became very difficult to distinguish from the destructive inflammation

If the disease was not recognised in time the child became blind, even if life was saved by dosing with cod liver oil Short of complete blindness the sight of the eye was sometimes saved, but was obscured by a permanent scar on the cornea Plate 25 shows a case of active xerophthalmia in an infant Plate 26 shows a photograph of a child who was cured of xerophthalmia at the expense of one badly scarred eye Plate 27 shows a child who also survived, but with total blindness following the infection of the anterior

References p 415



Plate 25 Xerophthalmia in a two year old Danish child The child appeared to be healthy except for a catarrhal infection and tightly closed eyes which were completely destroyed The conjunctiva were swollen and red both corneas were necrotic and there was pus in the anterior chambers The child survived but became totally blind (Bloch 1924)



Plate 6 Cured xerophthalmia The disease first developed when the infant was 3 months old and after 2 months of feeding with oatmeal gruel Keratomalacia led to total necrosis of the left cornea but the sight of the right eye was saved after treatment with cod liver oil and milk (Bloch 19 1)

chambers of the eyes with pus In untreated cases death results from spread of infection, or from pneumonia



Plate 27 Cured xerophthalmia Keratomalacia developed in both eyes at an age of 2 months after a diet of oatmeal gruel The child's life was saved by changing the diet to human milk but the sight of both eyes was lost The left eye was wasted and the right staphylomatous (Bloch 1921)

Statistical studies by Blegvad[1] of the number of cases of xerophthalmia recorded in Denmark indicated a rapid increase in the incidence of the disease between 1909 and 1916 which was followed by a dramatic fall after the cause of the disease had been discovered (Fig 28) There was

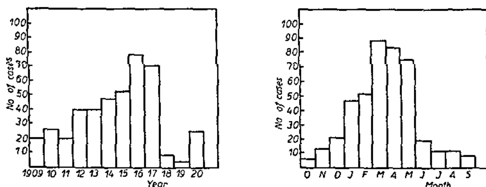


Fig 28 The number of cases of xerophthalmia reported in Denmark each year between 1909 and 1920 Note the dramatic fall in the incidence of the disease in 1918 after the importance of vitamin A had been appreciated (Blegvad 1923)

Fig 29 The occurrence of cases of xerophthalmia in Denmark, during the period 1909–1920 according to the season of the year Note the much greater incidence of the disease in winter and early spring than in summer and autumn (Blegvad 1923)

References p 415

of cows and margarine were incriminated as factors conducive to xerophthalmia About 21% of the patients died in spite of treatment Of those who survived 27% became completely blind, 24% were partially blind in both eyes, 35% preserved good sight in one eye, and 14% good sight in both eyes Over the period covered keratomalacia was reported in only 19 adults, and of these 18 were inmates of lunatic asylums

A point of great interest, which emerges clearly on reading Bloch's papers 30 years after their publication, is the influence of systemic disease in precipitating the ocular lesions Very often the appearance of xerophthalmia was preceded by a long period of failing health, with debility, diarrhoea and infections Three main types of preliminary illness were recognised, although the symptoms of different types were often combined The first two types were associated with a diet unduly rich in carbohydrates, and were differentiated by the child becoming either atrophic or oedematous (Plates 28

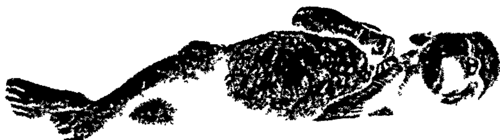


Plate 28 *Keratomalacia in an infant which had subsisted on a diet unduly rich in carbohydrates* Bloch s first type of predisposing disease Note the atrophy pot belly dry scaly skin and characteristic position of the hands (Bloch 1921)

and 29) These conditions were stated to be closely similar to those seen in the ' mehlnahrschaden of Czerney and Keller [15], which many modern authorities are inclined to identify with the "kwashiorkor" of Cecily Williams [16] A photograph by Bloch of an atrophic bellied Danish infant, with dry scaly skin (Plate 28), is certainly reminiscent of similar pictures of the effect of protein deficiency in African infants

The third type of preliminary illness was less severe (Plate 30) At first sight the children seemed almost normal apart from the eye complaint, but on closer inspection they were seen to be backward in development, weak, thin and anaemic Before the specific role of vitamin A was recognised

Bloch considered that this condition was due to lack of fat in the diet The description *dystrophia alipogenica* therefore seemed appropriate and a resemblance to the hikan which Mori [11] had studied in Japan was pointed out It seems probable that cases of this type occurred when the diet was deficient in vitamin A but adequate in protein as with a regime composed mainly of skimmed milk Cases of the two former types presumably occurred when the skimmed milk was combined with excessive amounts of sugar or starch



Plate 29 Oedema thout xerophthalm a n a 3 months old infant which had subs sted on a d et rich in carbohydrates Bloch s second type of condition pred sposing to xerophthalm a In th s case ho e er e les ons had been avo ded by the inclus on of a little butter fat in the d et (Bloch 19 1)

Xerophthalmia in India Impoverished classes in India have often to subsist on food even worse than that available to the infants studied by Bloch since no form of milk may be available Aykroyd and Krishnan [17] examined the eyes of over 400 children aged 1 12 years who had been removed from famine areas and housed in camps Their diet consisted of two kinds of lentils egg plant and bitter gourd As mentioned in the preceding chapter the average carotene intake was about 1100 I U per day Ocular abnormalities were seen in 27% of the children In 70% of the affected cases the abnormality amounted only to dryness smokiness or wrinkling of the conjunctiva The remaining 30% of positive

References p 415

cases however had reached a more advanced stage with definite Bitot s spots

Kirwan Sen and Biswas[18] examined nearly 15 000 patients who visited the Calcutta eye infirmary and found evidence of vitamin A deficiency in about 3% of these patients The ocular affections had been preceded by diarrhoea in 26 cases by colitis in 3 cases by jaundice in 5 cases and by hyperkeratosis of the skin in 6 cases Pigmentation of the conjunctiva or Bitot s spots were not accepted as sure signs of current vitamin A deficiency



Plate 30 Keratomalacia with total necrosis of the corneas in a two-year old child who had been fed on a diet of centrifuged milk Bloch s third type of condition predisposing to xerophthalmia The child was weak and dystrophic but symptoms ascribable to protein deficiency were absent (Bloch 1921)

since 70–80% of the patients showing these abnormalities had normal dark adaptation The severity of the injuries ranged from hemeralopia without visible injury of the eye up to rupture of the cornea and blindness Light cases responded to oral dosing with cod liver or halibut oil but severe cases were given massive injections of a vitamin A concentrate Treatment was successful in a high proportion of cases and even in completely blinded patients the general health was much improved

In another communication Biswas[19] expressed regrets that patients coming to the eye infirmary for the treatment of xerophthalmia had often been delayed in the general hospital for the treatment of gastro intestinal

or liver diseases Very often therefore they arrived too late for their sight to be saved Thus out of 215 cases treated by Biswas 127 arrived with advanced keratomalacia Of the more severe cases more than 70% were children under 5 years old Deficiency of vitamin A was due sometimes to a defective diet notably in poverty and sometimes to inefficient absorption or increased utilisation of the vitamin Ocular lesions occurred most often in the rainy season in parallel with the high incidence of diarrhoea dysentery and colitis The progress of ocular lesions could be divided up into six stages (1) defective dark adaptation (2) keratinisation and the production of Bitot s spots (3) diminished sensitivity of the cornea (4) degeneration of the cornea (5) breakdown of the cornea with inflammatory reaction in the eye (6) blindness It is interesting that these careful investigations included confirmation of Blegvad s finding that the incidence of xerophthalmia is greatly influenced by the season of the year

Xerophthalmia in the Fast Indies and Malay

Hemeralopia and xerophthalmia were noticed among the inhabitants of the East Indies by Wille [20] in 1919 soon after the first descriptions of Bloch s observations in Denmark Later the occurrence of keratomalacia was reported by De Haas [21] An important investigation by Meulemans and De Haas [22] followed in 1936 with the vitamin A content of the mother s milk as its central theme As mentioned in Chapter 21 the milk of European women in Batavia was much richer in the vitamin than the milk of the native women or of women of Chinese origin There was also evidence that the milk of badly fed mothers in the poorer classes was correspondingly low in vitamin A

In 1938 the first chemical estimations of vitamin A in the blood of patients with xerophthalmia were made by De Haas and Meulemans [23] For 11 infants with xerophthalmia including three who were blinded a very low range of values was found Thus six gave zero readings and the others only 4 6 8 22 and 31 i u per 100 ml Low values were also found for carotenoids In a group of 16 older children aged 1 7 years xerophthalmia was accompanied by zero readings for the blood vitamin A in 13 instances and by values of 2 14 and 38 i u in the other three cases It is regrettable that at the time of these studies no apparatus more sensitive than the Lovibond Tintometer was available for the estimation of vitamin A There seems no doubt however of the reality of the very low levels Thus in the Sheffield experiment on vitamin A deprivation plasma levels of under 40 i u per 100 ml were associated with defective dark adaptation (Chap 29) In the group of Indonesian infants a diet of sweetened skimmed milk was the most common cause of the deficiency Three of the infants however had been breast fed and these included one who was blinded Previous findings

References p 415

on the low vitamin A contents of the breast milk of native women were thus given striking support The older children had been subsisting mainly on rice and vegetables

In 1941 De Haas, Posthuma and Meulemans [24] published an interesting statistical survey of the incidence of xerophthalmia in children admitted to the Central Civil Hospital in Batavia Between 1935 and 1939 at least 353 cases were observed, amounting to nearly 5% of the total number of patients admitted Of the affected children 40–45% became either totally blind, or blind in one eye Diseases associated with the eye lesions included pneumonia, dysentery, dyspepsia and enteritis, pyuria, nutritional oedema, and worm infections About 75% of the affected cases were in an advanced state of dystrophy The mortality rate, however, was about the same, at 30–35%, for children admitted to the hospital either with or without xerophthalmia Sweetened skimmed milk was again incriminated as the most important cause of xerophthalmia, but about a quarter of the affected infants up to 2 years of age, had been inadequately fed at the breast

Van Veen and Lanzing [25] suggested that an incorrect distribution of food in the family, with an insufficient consumption of vegetables by the children, might be an important cause of vitamin A deficiency In Perak Adam Thomson [26] observed photophobia corneal pigmentation and often Bitot's spots in Malay children who had complained of night blindness The symptoms responded slowly to oral treatment with vitamin A, but more rapidly when oral treatment was reinforced by a single massive injection of a vitamin A concentrate

EVIDENCE OF PARTIAL VITAMIN A DEFICIENCY

In Great Britain and the U S A cases of xerophthalmia are very rare Thus in answer to a questionnaire circulated in 1933 by Hess and Kirby [27] eleven out of forty one American eye specialists denied having seen a single case. Typical of other replies was a record of five cases during fifteen years of practice, or the observation of the lesion only in one man who was subsisting mainly on potatoes According to this survey clinical hemeralopia was equally rare It seems probable that similar answers would be received to a questionnaire circulated in Britain Although epidemics of hemeralopia have occurred in Newfoundland and Labrador [28, 29], these districts seem distinguished from most parts of Canada and the U S A by the rigours of their climate, and also by the poverty of their inhabitants

Lack of evidence of complete deficiency, however, does not necessarily imply that the intake of vitamin A must always be fully adequate It seems reasonable to assume that states of partial deficiency can pertain in which

xerophthalmia remains in stages too early for easy observation, and the deterioration in dark adaptation is not sufficiently pronounced to be noticed by the subject Careful clinical and laboratory studies, however, should be capable of revealing the defects, and should therefore provide means of studying the vitamin A status in dietary surveys

Slit lamp examinations The detection of early xerosis of the conjunctiva was studied by Wiehl and Kruse [30] by means of slit lamp microscopy In New York school children the incidence of "avitaminosis A" appeared to be about 90%, which seemed to be suspiciously high Kodicek and Yudkin [31], who made similar studies in England, were more cautious in their conclusions Thus they stressed the difficulty of fixing the limits of normal variation in the conjunctiva and of deciding the causes of abnormalities Robertson and Morgan [32] studied a group of 40 young nurses, all of whom had thickening of the bulbar conjunctiva According to Kruse, therefore they must all have suffered, at some time, from deficiency of vitamin A Except in one instance however, no evidence of improvement could be found after half the nurses had been given massive doses of vitamin A for periods of up to two years In this study thickening of the conjunctiva appeared to be related to indulgence in outdoor sports

Dark adaptation measurements Studies of the efficiency of dark adaptation have been more informative, although the interpretation of results has not always been easy According to Jehgers [33] dark adaptation was abnormal in 35% of a large group of American medical students In 12% of the students moreover, deficiency was also indicated by subjective night blindness, photophobia or follicular hyperkeratosis The deficiency was attributed to the missing of meals or poor choice of diet. Doses of 4000 i u of vitamin A were necessary for protection, and the abnormalities responded to dosing A surprising finding, in the light of subsequent experience was a deterioration of dark adaptation, in Jehgers himself, after only 6 days of restriction to a diet low in vitamin A The liver reserves of vitamin A which should have been substantial after subsistence for the previous three months on a diet rich in vitamin A and carotene, were apparently unable to prevent the deterioration

Maitra and Harris [34] found subnormal dark adaptation in 20–36% of the pupils in various English elementary schools In pupils of a "public" school, presumably of a higher social status, there were very few cases of poor adaptation Poor dark adaptation was improved in pupils who were dosed with vitamin A, but remained abnormal in pupils who were left undosed Similar conclusions were reached by Harris and Abbasy [35] who found that it was possible, by studies of dark adaptation, to pick out from a class those pupils who were receiving special supplements of milk It was

References p 415

emphasized that poor dark adaptation can only be accepted as an indication of vitamin A deficiency if an improvement results from subsequent dosing with the vitamin

Steininger and Roberts[36] were much less confident about the value of dark adaptation measurements as a guide to the vitamin A status Findings in repeated examinations in the same individual were very variable In groups of children, who had once shown poor adaptation, many failed to improve when dosed with vitamin A, or improved even although they had not been dosed One subject, studied over a year, maintained a fairly constant trend in adaptation in spite of variations in the vitamin A intake from 100 to 100,000 i u daily (In retrospect, however, we may remember that reliability of the "biophotometer', used for measuring dark adaptation in this work, was not universally accepted)

Vitamin A deficiency with beri-beri

In 1940 Steven and Wald[37] carried out an interesting survey in Newfoundland and Labrador, where both hemeralopia and beri beri were endemic Vitamin A deficiency was diagnosed whenever adaptation, irrespective of its original efficiency, could be improved by a fixed extent after dosing with vitamin A In about 350 subjects, of whom about half were children, the incidence of such "vitamin A labile thresholds" was 9 7% For clinical night blindness the incidence was 3%, but there were no cases of xerophthalmia About half the subjects deficient in vitamin A also had beri-beri but treatment of the beri-beri with yeast had no effect on dark adaptation Both vitamin A deficiency and beri-beri had their highest seasonal incidence from February to March If infants were excluded both diseases occurred more often in adults than in children, with 26 as the average age for vitamin A deficiency and 40 for beri beri

Dark adaptation in relation to the level of vitamin A in the blood

At this stage reliable methods became available for the estimation of vitamin A in blood Attempts were made, therefore, to correlate the results of such estimations with dark adaptation measurements In the preceding chapter we have seen that there is a correlation at least to the extent that levels of under 40 i u per 100 ml of plasma, resulting from prolonged deficiency, are associated with defective adaptation

In studies on a large number of American children Josephs Baber and Conn[38] found very wide ranges both for the plasma vitamin A and for the efficiency of dark adaptation, but could trace no consistent relationship between these two variables When the children were arranged in four groups, according to their social circumstances and the quality of their diets, it was found that the averages for carotene in their blood were

correspondingly graded An inferiority in blood vitamin A however could only be noticed in the lowest group in which the efficiency of dark adaptation was also low

We may perhaps consider in the light of our present knowledge that Josephs and his colleagues were expecting too much in seeking closely graded relationships between the adequacy of the diet vitamin A in the blood and the powers of dark adaptation Their careful work deserves attention however for its evidence both by chemical and physiological tests that a poor diet even in a community which must be considered prosperous on international standards can cause a concealed subnor mality of vitamin A in the worst fed section of the population The absence of xerophthalmia and of other obvious signs of acute deficiency does not therefore necessarily imply that all is well with the vitamin A status

Yarbrough and Dann [39] also failed to find any general relationship between blood vitamin A and dark adaptation in a study on about 150 normal subjects

Vitamin A in gerontology Interesting observations on vitamin A de ficiency in old age have been reported by Kirk and Chieffi [40] Twenty three infirmary inmates aged about 70 years and of both sexes were picked out for study because of their low levels of vitamin A as found in a preliminary survey Repeated estimations confirmed that low levels of vitamin A still pertained with an average of about 17 i u per 100 ml Clinical examination showed thickening of the conjunctiva in seven of the patients blepharo conjunctivitis in sixteen and toad skin in thirteen When daily doses of vitamin A acetate were given the average concentration in the plasma rose rapidly to about 150 i u and remained at this level during the year for which dosing was continued In many cases the abnormalities in the eyes and skin were slowly improved until in some cases complete cures had been achieved Thus in contrast to the findings for younger subjects by Robertson and Morgan [32] complete normality was restored in all the seven cases of thickening of the conjunctiva Dark adaptation was greatly improved in four subjects but remained unchanged in the others

When dosing of these old subjects was stopped the level of vitamin A in the plasma fell rapidly to an average of about 53 i u but this level was maintained throughout observation for a further seven months In some instances the abnormalities which had been cured by therapy tended to reappear The findings in this American work may be reinforced by obser vations in England (Chap 29) that both life in an infirmary [41] and old age [42] may adversely affect the vitamin A status

References p 415

Bitot's spots These marks on the conjunctiva have already been mentioned but deserve fuller description as a sign of deficiency which has not been sufficiently acute to provoke frank xerophthalmia and keratomalacia According to Bitot the abnormality appears as an "assembly of glistening white points, which produce a pearly or silvery spot beside the transparent cor[illegible]

of the night bl[illegible]

ployed Usually the spots were on the outer (temporal) side of the eye, and formed a triangle with a base of about 5 mm, and sides 8 mm long, which was placed with its base near the cornea (Plate 31) Occasionally the spots were made up of small lines instead of points In a few cases spots were also found on the nasal side of the eye Bitot forestalled the results of later systematic research on vitamin A deficiency by recognising that the spots were due to a "special squamous production of the conjunctival epithelium", rather than to an invasion by a foreign agent



Plate 31 Bitot's spots in a Ceylonese subject (Nicholls 1939)

Similar spots have since been reported by many observers, but their descriptions suggest that the abnormality does not always take quite the same form Nicholls and Nimalasuriya [43] noticed conjunctival abnormality in several hundred Ceylonese children As early changes they found thickening and pigmentation of the conjunctiva of the sclerotics One or more of the blood vessels running towards the cornea stood out as though dilated and capillaries could be seen dipping vertically into the thickened epithelium Later the thickening and pigmentation increased, and heaped up accumulations of epithelial cells arose These white accumulations stood out in relief against the pigmented background, and were usually striated In [illegible]s resembled dabs of chalk which had been [illegible]cription is perhaps not very widely different [illegible]s remembered the effects are being compared in white and coloured subjects, and under different climatic conditions It [illegible] Nimalasuriya seldom found that

Other workers have described conjunctival spots which had the appearance of foam, either white [44, 45] or yellowish in colour [46]. Snell [9] likened the spots to bubbles, which could be obscured by pressure. Nicholls and Nimalasuriya [43] suggest that the exact appearance of the spots may depend on their speed of formation. Prolonged mild deficiency will slowly produce pearly white spots as first seen by Bitot, but more acute deficiency will rapidly cause loose accumulations of degenerated epithelium, and so cause a foamy appearance. The spots observed in their own studies tended to be very chronic, and large doses of vitamin A were required before they could be made to disappear.

Skin Lesions in Primary or Secondary Vitamin A Deficiency

The central cause of the abnormalities which have been described in the preceding sections may be accepted as a deficiency of vitamin A, or carotene, from the diet. We must remember, however, that infectious diseases can precipitate xerophthalmia in children who are already imperilled by a diet low in the vitamin. Defective dark adaptation may be caused not only by deficiency of vitamin A, but by old age or illness. Before we proceed to discuss the relationship of skin lesions to the vitamin A status the importance of such complicating factors requires further emphasis. Firstly we must realise that when skin lesions occur in malnourished populations it is often difficult to decide whether absence of vitamin A, or of some other nutrient, is the essential cause. Thus it may be difficult to incriminate vitamin A deficiency when it is known that rather similar lesions can be caused by deficiency of nicotinic acid or vitamin C. Secondly it seems well established that certain skin lesions will sometimes respond to vitamin A therapy even although there has been no dietary deficiency. We can reasonably accept, therefore, the hypothesis of "conditioned" or "secondary" deficiency of vitamin A. As far as possible we must attempt to discriminate between skin lesions due to "dietary" and "secondary" deficiency.

Hyperkeratosis in dietary vitamin A deficiency. The skin lesion most characteristic of vitamin A deficiency takes the form of hyperkeratosis of the hair follicles, which become hard and protuberant. Plate 32 shows papules on the arm of one of the volunteers in the Sheffield experiment on vitamin A deficiency [47]. When the lesion is severe the appearance of the skin amply justifies the description "Phrynoderma", or "toad skin" first applied by Nicholls [48, 49]. In some cases the skin between the protruding follicles appears more or less normal, but in others it becomes dry and scaly. Rao [50] has published photographs by R. E. Wright which indicate that scales may sometimes become more prominent

References p. 415

than the papules (Plate 33) These scales are cracked to form a diamond shaped pattern which has doubtless suggested the description Giraffe skin"



Plate 32 Follicular hyperkeratosis on the outer aspect of the upper arm of a volunteer who had been deprived of vitamin A for about a year Slight enlargement of the hair follicles however was also observed in volunteers in the same invest gation who were dosed with carotene (Hume and Krebs 1949)





Plate 33 Dry and atrophic sk n in a man aged 20 years w th keratomalacia Note the cracked appearance of the skin and the scales covering the body Front and s de views of the same subject (Rao 1937)

The association between xerophthalmia and a dry scaly skin was noticed by de Gouvea in 1883 [10] and later by Mori [11] and Bloch [12 14] In groups of *adult Chinese suffering* from keratomalacia Pillat [51] and Frazier and Hu [52] found that the skin was dry and covered with flakes of horny epithelium

They also observed hair
follicles with cornifi and
legs The condition vita
min A Loewenthal [53] noticed similar abnormalities in East African prisoners The papules were present in all cases having xerophthalmia or defective dark adaptation and were cured by cod liver oil without any other treatment Nicholls [48, 49] reported that symmetrical papular eruptions with the skin dry and scaly were common among the inmates of Ceylonese prisons The eruptions were associated with xerophthalmia and sometimes with dysentery and neuritis

In Britain Goodwin [54] observed a single case with papular eruptions which were cured by improvements in the diet and dosing with cod liver oil Youmans and Corlette [55] had similar experience with twenty cases in America Lehman and Rapaport [56] studied nine cases among American children in families receiving poor relief and confirmed their low vitamin A status by measurements of dark adaptation Papules containing broken hair or coiled unerupted hairs were found most often on the legs and arms but sometimes also on the buttocks back neck and face The response of the skin lesions to treatment was slow and maximum improvement was only secured after massive doses had been given for 2–4 months The restored downy growth of hair in the affected areas gave a striking indication of the efficacy of the treatment It was suggested that the conditions known as *keratosis pilaris lichen pilaris lichen spinulosus ichthyosis pilaris* etc are all merely descriptive terms for the papules which result from deficiency of vitamin A

Other workers however have advised caution in the diagnosis of skin diseases before vitamin A is accepted as the cause Fitzgerald Moore [57] has emphasized the importance of distinguishing the effects of vitamin A deficiency from those of pellagra Wiltshire [58] observed hyperkeratotic follicles in scurvy Stannus [59] questioned the specificity of vitamin A deficiency as the cause of phrynoderma and similar conditions which he considered might be identical with *keratosis pilaris* as seen in Britain In his opinion factors such as the exposure of the skin to air and irritation must not be overlooked

Skin lesions in conditioned vitamin A deficiency

In the clinical observations which have just been described the diet was known or suspected to be deficient in vitamin A We must now turn to skin diseases which occur in patients whose diets appear to be adequate but who nevertheless may respond to massive dosing with the vitamin A conditioned defect in vitamin A metabolism therefore seems probable We may deal firstly with certain rare diseases in which the skin

References p 415

lesions are usually associated with low levels of vitamin A in the blood plasma Secondly we come to common conditions in which benefit from dosing has been reported, although there is no evidence that the level of the vitamin in the blood is low

Darier's disease The essential lesion in this rare disease is a keratosis of the mouths of the follicles, which form firm pinheaded sized confluent papules These soon become covered by crust, and later produce vegetating papillomatous growths The eruptions may be confined to the face or neck, or may spread over the whole of the body The usual description "*keratosis follicularis*" is virtually identical in meaning with the "*hyperkeratosis follicularis*" usually applied to "toad skin", and with "*keratosis pilaris*" and "*dyskeratosis follicularis*" which seem to be loosely applied to any condition with hardening and elevations round the hair follicles Apparently Darier's disease is distinguished from other forms of follicular hyperkeratosis by the intensity of the hyperkeratosis, the encrustation, and by products of cell degeneration known as "corps ronds" There is no history of dietary deficiency, and the disease follows a chronic course which is little affected by conventional forms of treatment

Peck *et al* [60–63] reported that in Darier's disease the level of vitamin A in the blood was low Treatment with massive doses of vitamin A (200 000 i u daily) not only increased the level in the blood, but in almost all cases had a favourable clinical effect Darier's disease therefore appeared to be due to hereditary or acquired weakness in the absorption of vitamin A, or in the conversion of carotene In support of this conclusion several other American workers [64–68] all reported on one case each, which usually improved after treatment with vitamin A The experience of Carleton and Steven [69], however, was somewhat inconclusive Dark adaptation was normal in all the cases at their disposal The blood vitamin A was low in only one out of four cases but two cases improved clinically when they were given moderately large doses of vitamin A (20,000 i u) Pettler [70] observed no clinical improvement in a single case when massive doses of vitamin A were given

In Britain six cases of Darier's disease were studied by Leitner and Moore [71, 72] In repeated estimations before dosing was commenced the blood vitamin A was found to be frequently, but not invariably low Thus the average values for the six cases ranged from 55 to 106 i u per 100 ml and the lowest recorded values for each case were 7, 20, 28, 55, 55 and 103 i u Carotenoids were within the normal range Dosing with 100,000–240 000 i u of vitamin A daily raised the level in the blood to maximum values of 560–1800 i u Assessment of the effects of dosing was complicated by simultaneous treatment with Grenz rays, which caused temporary improvement in all cases The combined treatment, however, produced a com-

plete cure in one patient (Plate 34) even in areas which were not irradiated Three patients were considerably improved but not cured The remaining two cases showed no benefit even although vitamin A was raised to high levels in the blood In one case the improvement secured during dosing was lost after dosing had been stopped When dosing was resumed the absorption of the vitamin was found to be defective as evidenced by the absence of any response in the level in the plasma Further evidence of defective absorption of vitamin A in Darier s disease was reported by Ruch Brunsting and Osterberg [73]



Plate 34 Darier s disease in a 34 year old man *Left* Before treatment Note the crusted hyperkeratotic papules which also occurred on the neck back loins and elsewhere *Right* After treatment for 6 months with daily doses of 240 000 i u of vitamin A The dosing was accompanied by irradiation with Grenz rays which had previously failed to effect a complete cure when applied alone (Leitner and Moore 1948)

Devergie s disease (Pityriasis rubra pilaris) In this rare chronic exfoliating skin condition the papules coalesce to form reddened scaling areas which sometimes spread over the whole body The course of the disease is chronic and irregular, with unexpected exacerbations and there is a tendency for the lesions to become generalised in the form of an exfoliating erythrodermia Cures were reported by Pettler [74] in two patients dosed with vitamins A B and D and also in a patient dosed with carotene [70] Arquello [75] claimed the cure of an aged woman by massive doses of vitamin A but Thomas [76] was unsuccessful

References p 415

with an aged man Brunsting and Sheard [77] cured defective dark adaptation in three pityriasis patients by doses of 150,000 i u of vitamin A daily The skin was improved in two of these cases, and remained stationary in the other Peck and Chargin [60] cured one patient with extensive lesions by dosing with vitamin A.

Weiner and Levin [78] found that in five patients with Devergie's disease both the blood vitamin A and dark adaptation were normal, but in spite of this evidence of an adequate vitamin A status dosing with vitamin A or carotene was beneficial. In a boy with the disease Cornbleet, Popper and Steigmann [79] recorded a low value for carotene, and concluded that the defect lay in the conversion of carotene rather than in dietary deficiency or defective absorption. Porter and Godding [80] observed defective dark adaptation in a single patient, which was cured, with considerable improvement in the skin lesions, by prolonged dosing with vitamin A

Leitner and his colleagues [81, 82] studied not only cases of their own but also the records of many previous cases The main factor in the transmission of the disease appeared to be congenital, with the frequent occurrence of several cases in the same family Estimations of vitamin A in the blood of two patients gave low values, but dark adaptation was not defective In a female patient the skin lesions were aggravated during menstruation, and at the same time there was some evidence of a lowering in the vitamin A level In the patients mentioned in his papers Leitner found that massive dosing with vitamin A was followed by considerable improvement in the skin lesions, but not by complete cures. Complete success was attained, however, in a further two patients who were treated later [83]

Ichthyosis (fish skin disease) This condition, which may occur as a secondary manifestation of an internal disease, is characterised by a scaling of the skin Rapaport *et al* [84] found that the skin lesions were associated with poor dark adaptation, and that both abnormalities were improved by giving large doses of vitamin A Peck, Glick and Chargin [62] observed low plasma levels of vitamin A, but could not agree that any improvement following dosing exceeded the usual seasonal range of variations Leitner and Moore [81] reported rather low levels of vitamin in the blood in two cases

Glazebrook and Tomaszewski [85] investigated the vitamin A status in a man with Hodgkin's disease, with severe injury to the liver and general ichthyosiform atrophy of the skin Night blindness had been noticed subjectively for nine weeks before the outbreak of skin lesions, and the level of vitamin A in the plasma was very low. A further indication of deranged vitamin A status was found in the secretion of small amounts of the vitamin in the urine (Chap 32) Later another case was described in which ichthyosis

was secondary to lymphosarcoma Vitamin A was low in the blood, and remained low in spite of heavy dosing. Considerable quantities of vitamin A were excreted in the urine, but nevertheless large reserves were found in the liver at autopsy. A fault in the mobilisation of the vitamin from the liver was therefore suspected

Common skin diseases Indications of a poor vitamin A status, such as low levels in the blood or defective dark adaptation, have been reported less frequently in common skin diseases than in the rare conditions which have already been mentioned Claims for effective vitamin A therapy, however, must prevent us from dismissing the possibility of conditioned vitamin A deficiency in some common types of skin abnormality.

Marchioni and Patel [86] claimed that vitamin A was low in the blood in eczema and psoriasis but not in furunculosis Schneider and Widder [87] studied nearly 300 patients with various skin diseases, and found an average for vitamin A which was 36% below normal, and for carotene which was 25% below normal A low average serum vitamin A was also found in a small group of skin patients by Kuhnau and Luniatschek [88] In contrast Leitner and Moore [81] reported about the same averages for both vitamin A and carotenoids in about 100 patients with common skin diseases and in the same number of control subjects Cornbleet *et al* [79] found that in patients with skin diseases the blood vitamin A level was always above the "lower normal limit".

Acne vulgaris. In 1941 Maynard [89] claimed that patients with acne were benefitted by vitamin A therapy Straumford [90] treated 100 acne cases with daily doses of 100,000 i u of vitamin A, and found that 80% were cured, although sometimes only after treatment for six months Saunders [91] treated himself with vitamin A for sinusitis, without success, but was surprised when pustular spots of *acne vulgaris* disappeared from his back for the first time for 21 years Obermayer and his colleagues [92, 93] were successful with vitamin A therapy in some cases, but not in others Lynch and Cook [94], however, found that vitamin A failed to produce any marked improvement

In view of the finding by Ruch *et al* [73] that vitamin A may be poorly absorbed in skin diseases Davidson and Sobel [95] tried the efficiency of an aqueous dispersion of vitamin A, which also contained other vitamins, in the treatment of acne Out of twenty adult patients, aged 14–26, who had been affected for 1–10 years, improvement was seen within 2½–5 months in 18 cases There were two complete cures, and seven "much improved" or "practically cleared up". Improvement usually started after 2–3 weeks of dosing, and in many cases the acne tended to return after dosing had

References p 415

been stopped Downing[96] observed good results with vitamin A therapy in acne with numerous comedones In Scotland daily doses of 100 000 i u of vitamin A for 3 months were found by Lahiri and Scandrett[97] to cure acne in about 80% of a group of 75 patients (Plate 35) In the other patients the treatment had to be continued for 4–6 months Before treatment the levels of vitamin A and carotene in the blood of acne patients were not significantly lower than those of normal subjects An indication of abnormal vitamin A metabolism however was found in the decidedly low responses in the level of vitamin A in the plasma which were observed after giving acne patients a single large dose of the vitamin



Plate 35 Acne vulgaris treated with vitamin A *Left* Before treatment *Right* After treatment for about 3 months with daily doses of 100 000 i u of vitamin A (Lahiri and Scandrett 1954)

Other common skin diseases Cures of nummular eczema by large doses of vitamin A were claimed by Gross[98] Czibor[99] also reported the value of vitamin A combined with vitamin D in eczema Defective absorption of vitamin A in various forms of eczema was reported by Spector *et al*[100] and by Agnese and Larkin[101] According to Mackay[102] the administration of cod liver oil to infants reduced the incidence of napkin rash and of certain other skin abnormalities

In the treatment of psoriasis surprising results in reverse of conventional expectations were reported in 1947 by Hoffman Lorenzen and Garfinkel[103] Out of eleven patients nine were cured or much improved by restriction to a diet low in carotene and vitamin A One patient who had been cured relapsed after dosing with carotene

The Range of Abnormalities Attributed to Avitaminosis A

From the foregoing sections we may conclude that the main known effects of primary or secondary deficiency of vitamin A in the human are (1) defective dark adaptation (2) xerophthalmia and associated abnormalities

and (3) probably various skin lesions It is perhaps remarkable that there has been no mention of nerve and bone lesions such as have been observed in experimental animals In this connection there seemed until recently, to be little actual evidence beyond a claim by Nicholls [104] that nerve degeneration in paretic Ceylonese children may have been due to deficiency of vitamin A We may recall however, that long before the discovery of vitamin B_{12} Mellanby [105] had the idea that vitamin A therapy might prove valuable in the treatment of sub acute combined degeneration of the cord He also suggested that deficiency of vitamin A might be a conditioning factor in convulsive ergotism, pellagra and lathyrism

Hydrocephalus A slender link between experimental and clinical findings in regard to nerve and bone lesions now seems possible through the recent findings of Bass and Caplan [106] on the occurrence of hydrocephalus in a human infant A normal infant, one month old, developed allergy to cows' milk, as indicated by the appearance of eczema A diet of soya flour and bananas was given and the provision of vitamin A was overlooked At an age of 8 months the child was anaemic and mentally and physically backward The corneas were blurred and had small ulcerations Hydrocephalus developed, with bulging of the anterior fontanel Fortunately for the child the omission of the vitamin was then realised and massive doses were injected As a result the child recovered from all its abnormalities within 10 days

Further evidence of secondary vitamin A deficiency The precipitation of xerophthalmia by fever or intestinal disorders has already been mentioned earlier in this chapter Brief reference must now be made to the effect of certain other diseases, and particularly liver abnormalities which have the same effect

Altschule [107] reviewed the early literature on the occurrence of xerosis in liver disease Thus more than 50 years ago, before the discovery of vitamin A, Thompson [108] described the development of keratomalacia in a young infant suffering from jaundice Bloch [14] observed xerosis and arrested growth in a jaundiced child The condition persisted at first even when cod-liver oil was given, but cleared up when bile appeared in the faeces It must be concluded, therefore ,that the absorption of vitamin A was defective in the jaundiced child

Haig, Hecht and Patek [109] observed faulty dark adaptation in cases of cirrhosis of the liver, which were mostly of alcoholic origin Wohl and Feldman [110] confirmed this finding and also observed that adaptation was defective in most cases of thyroid disease, whether hypothyroidism or thyrotoxicosis Ezickson and Feldman [111] reported an association between urolithiasis and defective dark adaptation

References p 415

For the discussion of conditioned subnormalities in the vitamin A status, as measured by chemical estimations rather than by pathological observations, we must wait until our next chapter.

The avitaminosis A spectrum. In the course of this chapter we have commenced with an account of the classical diseases which are due without doubt to deficiency of vitamin A We have finished in discussing diseases in which a disturbed vitamin A status may still be considered of questionable significance, with some authorities accepting, and others denying its importance.

It is inevitable that many readers will still be sceptical, for example, over the value of vitamin A for the treatment of such a common disease as acne There is no clear evidence that this abnormality arises from a dietary defect, or that the level of vitamin A in the body is low. Can we accept evidence of the importance of vitamin A therapy merely from reports of a high percentage of cures, but some only partial, in a condition which is anyhow subject to spontaneous remissions?

The author has no right or qualification to sit in judgment, but he can at least emphasise the difficulty that such a judgment must involve, and the care which must be exercised against prejudice for or against the value of vitamin A therapy.

The difficulty arises from the ill-defined gradations which make up the spectrum of abnormalities which have been reviewed in this chapter Thus a frank case of xerophthalmia which will respond to vitamin A therapy will probably be accepted as a clear case of primary vitamin A deficiency, even if the eye lesions may have been preceded by fever or diarrhoea. If the xerophthalmia is associated with liver disease, however, and responds only when the poor absorption of the vitamin has been overcome by massive therapy, then perhaps the case can better be classified as secondary vitamin A deficiency.

A case of phrynoderma will be accepted as primary deficiency if the diet has been low in vitamin A, and there is a good response to therapy. In Darier's disease not all cases will respond, but there is evidence that the absorption of vitamin A, and its level in the blood, are often defective. A secondary deficiency conditioned by some systemic effect, possibly hereditary, may reasonably be inferred

In acne both the dietary intake of vitamin A and its level in the blood are usually normal, but absorption may be poor, and many cases appear to [illegible] vitamin A

m [illegible]

Fi [illegible]

decline in the level of vitamin in the blood (Chap 21). These changes

appear irrespective of the general nature of the diet, and disappear after parturition. The administration of doses of vitamin A, sufficiently massive to give a significant improvement in the dark adaptation while the subject is still pregnant, may seem unneccessary, and perhaps even unadvisable. In the author's opinion future research may reveal the importance of

concentrations, in tissues which normally contain only traces Thus the subcutaneous fat deposits can become loaded with vitamin A It has also been proved that vitamin A, in massive doses can effect the structure of the skin, both in normal rats (Chap 28) and in mice suffering from an hereditary form of hyperkeratosis [112]

It must be realised of course, that such an unnatural surfeit of vitamin A, in the subcutaneous tissues, will not necessarily restore the correct sequence in the forming, maturing and shedding of skin The increased concentrations of the vitamin, however, may well be expected to counteract the influence of a disease causing hyperkeratosis by stimulating the production of immature nucleated cells. Between the two opposing forces the skin, and other diseased tissues subjected to the same influences, may sometimes contrive to heal themselves.

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CHAPTER 32

The Vitamin A Status in Diseases not Attributed to Vitamin A Deficiency

The preceding chapter has been concerned with diseases which are caused or are at least believed to be caused, by vitamin A deficiency, either dietetic or secondary in origin We must now reverse our direction of approach, and study the effects of disease on the vitamin A status Moreover in the preceding chapter our interests lay mainly in abnormalities, such as xerophthalmia and hemeralopia, which could be considered characteristic of avitaminosis A Our field must now be extended to include all types of disease We must try, however, to distinguish between those diseases in which the vitamin A status is profoundly disturbed and others in which the status is relatively little affected We must recognise that in certain common diseases the stress on the vitamin A contents of the body may be so severe as to raise fears of secondary avitaminosis

There are at least six ways in which the vitamin A status can be affected by disease (1) The intestinal absorption of the vitamin, or of its provitamins may be defective Loss of preformed vitamin A, or of amounts of pro vitamin in excess of the normal proportion, may occur in the faeces (2) The level of vitamin in the blood may be greatly reduced This reduction is particularly characteristic of fever, but may also occur in other conditions (3) Urinary excretion of the vitamin, sometimes in substantial amounts may occur in certain diseases (4) The stores of vitamin in the liver may be reduced, or sometimes completely lost (5) In diabetes, and possibly in hyperthyroidism, the metabolism of the vitamin may be so affected as to increase its storage in the liver (6) In nephrosis and possibly in other diseases, the level of vitamin A in the blood may be greatly increased

It should be clearly understood that in any particular case several of these abnormalities may coincide Moreover some degree of interrelationship between the abnormalities must obviously be expected Thus a low level of vitamin A in the blood plasma may be associated on the one hand with excretion in the urine, and on the other with reduced liver reserves Unfortunately few investigators have been able to study the effect of a par-

ticular disease on vitamin A metabolism from all possible angles In order to gain as complete a picture as possible therefore it is often necessary to combine the results of different workers Thus one group of investigations has been concentrated on the levels of vitamin A in the liver in various diseases and another group on the levels in the blood The correlation of results by different workers is not always easy but much important infor mation may certainly be gained in this way

The Vitamin A Contents of the Liver Blood and Excreta as Affected by Disease

For convenience we shall first deal separately with the effects of disease on the levels of vitamin A in the liver blood urine and faeces respectively Later the information under these separate headings will be combined for the special discussion of those diseases in which the metabolism of vita min A is most notably influenced

Vitamin A in the liver The great variations in vitamin A reserves between individuals even in health have been emphasised in Chapter 29 In studying the effect of disease therefore it is necessary to collect data on groups of subjects and to avoid the injudicious use of simple averages in comparing data obtained for different groups It will be recalled that for cases of accidental death the author resorted to the procedure of arranging the individual values in ascending order and then dividing them up into three equal sub groups low middle and high The mean of the middle third of the cases was quoted as the median for the group

The two largest investigations of liver reserves in disease each on about 1000 specimens were made before the 2nd World War by Wolff[1] and Moore[2] Over a period of 20 years less extensive studies were also made by other investigators[3–16] Comparisons between the results of the different workers are made difficult by variations in the grouping of diseases and by some uncertainty that the factors used to convert results obtained by the antimony trichloride method into international units have always been correct The author may perhaps be pardoned therefore if he relies on his own findings to provide a central viewpoint from which the results of other workers may be profitably surveyed

Table 49 gives the median reserves found in groups of subjects usually within the age limits of 15–59 years who had died from various diseases In comparison the median reserve for subjects who died within 7 days of an accident is quoted It will be seen that medians above the level in accidental death were found only in diabetes and thyroid diseases Below

References p 440

the level of 220 i u per g found in the accident group the reserves in the various other disease groups decreased over a wide spectrum with extremes of 170 and 19 i u Since the data were not amenable to conventional statistical treatment too much importance should certainly not be attached to the exact order in which the diseases have fallen Broadly speaking however we may conclude that the diseased subjects taken as a single class tended to have reserves of vitamin A which were *only* about half those found in accidental death Among the different diseases moreover some could be recognised as having ranges of reserves only slightly below the level in accidental death whereas in others the reserves were very low indeed Thus the median reserve in hernia and allied conditions was 160 i u per g as compared with only 25 i u in chronic nephritis We shall see later that the combined experience of many investigators has fully confirmed the tendency for certain diseases to be associated with particularly low reserves of vitamin A

TABLE 49

MEDIAN VITAMIN A RESERVES IN THE LIVERS OF HUMAN SUBJECTS AGED 15–59 YEARS WHO DIED FROM ACCIDENT OR FROM VARIOUS DISEASES (MOORE 1937)

Cause of death	*No of cases*	*Vit A reserves i u /g (liver)*	*Cause of death*	*No of cases*	*Vit A reserves (i u /g liver)*
Thyroid diseases	9	310	Syphilitic aortitis	27	90
Diabetes	15	300	Septic endocarditis	33	90
Accident	40	220	Thrombosis embolism	23	89
Poisoning	13	170	Bronchiectasis	12	82
Hernia and some other			Subacute nephritis	12	75
conditions	10	160	Peritonitis	12	75
Blood diseases	31	130	Enteritis colitis	11	74
Cerebral haemorrhage	26	120	Head spine infections	52	73
Gastric and duodenal			Pneumonia	22	63
ulceration	43	110	Empyema	12	60
Appendicitis	19	110	Valvular heart disease	56	60
Gall bladder diseases	13	110	Abscesses	35	51
Cancer	76	110	Prostate diseases	23[a]	40
Nerve degeneration	13	110	Chronic nephritis	48	25
Coronary disease	20	100	Urinary infections	13	19
Tuberculosis	26	96			

See also Appendix Table 62 p 584 [a] All ages

The data collected in Holland by Wolff[1] are given in Table 50 It will be seen that in this work the average found in accidental death acute diseases and chronic diseases were only slightly different There was therefore a disparity with the author s finding that the reserves in disease tend to be substantially lower than in accidental death If consistency of calibration between the two studies can be assumed which is by no means certain

the difference would appear to reside in the ranges found in accidental death and not in those found in disease Certain comforting points of agreement between the results of the two investigations will be mentioned later

TABLE 50

AVERAGE VITAMIN A RESERVES OF THE LIVER IN FATAL CASES OF ACUTE AND CHRONIC DISEASE[a] (WOLFF 1932)

Acute diseases			*Chronic diseases*		
Disease	*No cases*	*Vit A i u/g*	*Disease*	*No cases*	*Vit A i u/g*
Yellow liver atrophy	3	88	Heart disease	102	98
Poisoning	2	336	Malignant tumours	159	104
Embolism	7	76	Pulmonary T B	66	120
Ruptured uterus	3	45	T B other forms	30	145
Apoplexy	19	135	Syphilis III and IV	33	61
Heart seizure	5	139	Kidney diseases	54	77
Haemorrhage	4	114	Diabetes	25	290
Ileus invagination	9	217	Brain and spine diseases	22	153
Peritonitis[b]	28	150	Prostate hypertrophy	8	179
Sepsis[c]	23	196	Leucaemia[e]	12	115
Erysipelas	9	77	Gastric ulcers[f]	16	229
Pneumonia lobar	47	127	Lung gangrene	5	152
Pneumonia broncho	47	108	Enteric fever	3	7
Measles	8	119	Liver cirrhosis	6	43
Whooping cough	12	74	Bronchiectasis	3	65
Diphtheria	13	230	Emphysema	3	170
Other infectious diseases	4	98	Exoph goitre	5	210
Otitis media[d]	19	150	Suppuration	8	129
Phlegmons etc	5	90	Foot gangrene	3	85
			Spina bifida	2	60
			Various	5	101
Total	267	135		570	124

[a] The average for 78 cases of accidental death was 147 i u per g with a range of 0–725 i u

[b] Including acute appendicitis

[c] One case gave a value of 1950 i u per g The average for the remaining 22 cases was 109 i u per g

[d] Including acute meningitis

[e] Including chronic anaemia

[f] Including duodenal ulcers

Vitamin A in the blood In 1938 a pioneer investigation on the effects of disease on the level of vitamin A in the blood was reported in a monograph by Lindqvist [16] As mentioned in Chapter 29 a Zeiss step photometer was preferred to the Lovibond tintometer in this work and unfortunately doubts arose later about the potency of a vitamin A concentrate which was used for its calibration It was clearly established however that th[illegible] le creased in [illegible] re-

References p 440

ously during recovery Further reference to this important work will be made in dealing with individual diseases

Another interesting study was described in 1946 by Tomaszewski and Dzialoszynski[17], who found low blood levels of vitamin A in various forms of liver disease. Popper *et al*[18] estimated vitamin A, and its distribution between free alcohol and esters, in nearly 200 hospital patients Their results, which are summarised in Table 51, confirmed the reduced levels of vitamin A in the blood in infections and in liver diseases In most diseases the proportion of vitamin in the esterified form, as found by phase separation was greater than in a control group

TABLE 51

AVERAGE CONCENTRATIONS OF VITAMIN A IN THE BLOOD PLASMA AND PERCENTAGES OF THE TOTAL VITAMIN A PRESENT IN THE ESTERIFIED FORM IN VARIOUS DISEASES (POPPER *et al*)

Diagnosis	*No of cases*	*Average total vit A i u /100 ml*	*Average % of esters*
Hospital controls	31	169	18 2
Cardiac disease	13	128	26 8
[illegible]	7	147	30 6
[illegible]	15	115	42 4
[illegible]	5	97	39 5
Malnutrition	4	74	56 0
Tuberculosis	11	96	44 1
Infections	2	133	34 5
Pneumonia	9	90	53 4
Pneumonia recovering	10	182	24 1
Nephritis	10	222	17 3
Nephrosis	6	188	40 0
Acute infectious hepatitis	14	113	38 1
Infectious hepatitis recovering	6	134	32 4
Acute toxic hepatitis	6	87	46 4
Cirrhosis without jaundice	12	89	47 9
Cirrhosis with jaundice	17	76	56 9
Obstructive jaundice	8	130	31 9

Vitamin A in urine Vitamin A is insoluble in aqueous solution, unless it is attached to protein or is carried by an emulsifying agent One would not expect, therefore, to find the vitamin in urine This expectation certainly proves correct in regard to normal human urine but substantial amounts are often excreted during illness Thus in 1936 Boller and Brunner[19] obtained positive results in the antimony trichloride method for 10 out of 41 pathological urines Half the patients excreting the vitamin had cancer Later Boller *et al*[20] published results on 321 cases Vitamin A was detected in the urine in icterus with closure of the biliary duct, chronic nephritis nephrosis, lobar pneumonia before crisis and

cirrhosis of the liver Large oral doses of vitamin A had little effect on the amounts excreted by the patients in their urine

Schneider and Weigand [21] confirmed the urinary excretion of vitamin A in cancer tuberculosis and chronic infections The excretion did not appear to be due to specific renal damage nor to be caused by the ingestion of liberal amounts of the vitamin It was claimed that the excretion only took place during hypovitaminosis C, and that it could be checked by giving ascorbic acid Lindqvist [22] found that in patients with pneumonia the excretion of vitamin A varied from 230 i u to 3100 i u daily before crisis and that it fell to zero after crisis Gaehtgens [23] reported the presence of vitamin A in the urine of 8 out of 31 cases of normal pregnancy After large doses of the vitamin had been given it was found in the urine in 19 of the cases

In chronic nephritis Hedberg and Lindqvist [24] observed constant or irregular excretion of vitamin A in all but two of 25 patients Large doses of vitamin A or of ascorbic acid had no effect on the abnormality Catel [25] confirmed the excretion of vitamin A in nephritis and made the surprising discovery that the vitamin is present in the urine of normal dogs (Chap 34) Of 26 patients examined by Grant [26] fifteen showed spontaneous excretion of vitamin A These included cases of carcinoma of the gall bladder cirrhosis of the liver icterus hemeralopia chronic nephritis nephrosis diabetes and pneumonia

The urinary excretion of vitamin A in skin disease was investigated by Marchionini [27] Out of 75 cases of non tuberculous skin disease vitamin A occurred spontaneously in the urine in seven cases and in fifteen after massive dosing with vitamin A Quite similar findings were made for tuberculous skin diseases and for syphilis No evidence of kidney damage could be found but reduced liver efficiency was often revealed by the galactose test Thiele and his colleagues [28–30] noticed that vitamin A was occasionally excreted by patients who were undergoing treatment for gonorrhoea or tertiary syphilis The excretion did not appear to be due to pre existing injury to the liver or kidneys but was frequent in patients who were treated by bismuth or by infection with malaria Both these treatments can cause impairment of the reticulo endothelial system which was considered to be the essential cause of the excretion of the vitamin

Studies by Lawrie McArdle Moore and Rajagopal [31, 32] included investigation of the quantitative significance of the urinary loss of the vitamin and of the mode of carriage of the vitamin in the urine Out of fifteen patients with pneumonia the urine contained vitamin A in thirteen cases in concentrations ranging from 6 to 480 i u per 100 ml The identity of the vitamin was confirmed by observation of its absorption band at 328 mμ and by

References p 440

a few biological tests with rats The daily output of vitamin was only recorded for four patients, but in one instance urine containing 160 i u per 100 ml was passed in such amounts as to cause a daily excretion of 3200 i u of vitamin A The concentration of vitamin in the urine in this case must have greatly exceeded the lowered level in the blood, and the daily loss certainly exceeded the requirement of vitamin necessary to maintain normal health In confirmation of Lindqvist the excretion of the vitamin ceased abruptly after the crisis had been passed

In chronic nephritis the same workers found small amounts of vitamin in the urine of six out of ten patients In 44 cases of other diseases urinary excretion of the vitamin was found in only three instances but the numbers of patients were too small to allow conclusions to be drawn as to the frequency of the excretion in individual diseases Catel's claim of the presence of the vitamin A in normal canine urine was confirmed, but in contrast to Gaehtgen's observations only a trace of vitamin could be found in one out of 30 specimens of urine collected from pregnant women No vitamin could be found in the urine of healthy subjects, even after massive dosing nor in the urine of normal or diseased rats and rabbits

It must be emphasised that the excretion of vitamin A into the urine appears to be a highly selective process, and not merely the reflection of a non specific leakage of lipoids In pneumonia the concentration of vitamin per ml in the urine may be ten times greater than in the plasma and the concentration per unit of lipoid must be at least a hundred times greater Preformed vitamin A is sharply separated from carotene, which does not pass into the urine in significant amounts. Lawrie and his colleagues found that some human urines containing the vitamin were quite clear Others were turbid, but sometimes filtration to complete clarity could be effected without removing the vitamin Protein, not necessarily heat coagulable was always present but no correlation was apparent between the amounts of protein and vitamin Thus no urinary vitamin A could be detected in a case of frank albuminuria The field certainly seems worth further investigation by methods exploiting modern techniques for protein separation

Vitamin A in faeces As stated in Chapter 14 the faeces of healthy subjects normally contain carotene, but not vitamin A An exception to this generalisation occurs, however, when infants are given heavy doses of the vitamin which may cause the passage of substantial amounts into the faeces [33] [34] This excretion is presumably associated with an efficiency of fat absorption below the level in adults Diseases characterised by defective fat absorption, such as coeliac disease, sprue and cystic fibrosis of the pancreas may be expected to cause the faecal excretion of vitamin A even in adults.

No comprehensive study centred on this topic seems to have been undertaken Barnes Wollaeger and Mason[35] however have included a comparison of the faecal excretion of vitamin A by patients with steatorrhoea and by control subjects in a paper mainly devoted to the effect of emulsification on the absorption of the vitamin Of their six patients five had non tropical sprue while the other had steatorrhoea associated with diabetes After massive doses of an oily solution of vitamin A had been given the percentages excreted in the faeces in the control and diseased groups averaged 12 2 and 56 3% respectively After similar doses had been given as an aqueous emulsion the excretions were 4 8 and 32 3% The influence of the defective absorption of the vitamin could therefore form in which it was administered

Abnormalities in the Vitamin A Status in Individual Diseases

The overlapping of lesions in disease and the fact that abnormalities in vitamin A metabolism are not associated with any one form of lesion makes it difficult to deal logically and systematically with the effect of disease on the vitamin A status It may be helpful however to recognise three groups of lesions which can be held responsible either singly or combined for most of the recognised abnormalities Our first group covers diseases in which the most obvious abnormality is a failure in the absorption of the vitamin often in association with defective fat absorption Secondly we must consider fevers, with the realisation that even artificially induced pyrexia will cause a fall in the level of vitamin in the blood[36] This group may well be extended to include chronic infections even when the temperature is not consistently high Thirdly we come to degenerative diseases in which disturbances in the vitamin A status occur in the absence of either fever or any obvious failure in the absorption of fats While no formal division of diseases into these three groups will be attempted in view of the overlapping which has already been mentioned we may consider individual diseases more or less in the order of the groups in which it seems most logical to place them

Coeliac disease This wasting disease in children which has been attributed to an abnormal sensitivity to the proteins of wheat or rye[37] involves defective fat absorption as a secondary effect Several investigations which have usually been based on the increases of vitamin A in the blood after dosing have shown that the vitamin is also less well absorbed by diseased than by normal children[38–40]

References p 440

Sprue Sprue is another disease involving a defect in the absorption of fat It affects adults, and takes the form of chronic inflammation throughout the intestinal tract. The cause is thought to be a nutritional defect, and this theory had been upheld by the response of the disease, and also of the macrocytic anaemia which accompanies it, to treatment with liver extract or with folic acid (pteroylglutamic acid), a member of the vitamin B complex As already mentioned Barnes *et al* [35] found that large amounts of vitamin A are excreted in the faeces by sprue patients Adlersberg and Sobotka [41] failed to observe any increase at all in the blood level of vitamin A after massive dosing with this vitamin, but increases were observed in patients who were enjoying remissions caused by the administration of liver extracts A similar improvement in the absorption of vitamin A was observed by Darby, Kaser and Jones [42] in a simple case of sprue after treatment with folic acid May [43] was unsuccessful, however, in the application of folic acid to five cases of well-established coeliac disease

Obstructive jaundice In Chapter 31 attention has already been drawn to early evidence that xerophthalmia and other indications of vitamin A deficiency may sometimes be developed as secondary effects of jaundice In later work the absorption of the vitamin, as measured by its increase in the blood after dosing, has been proved to be defective in this condition [44, 45] The importance of bile for the absorption of the vitamin has already been discussed in Chapter 18

Pancreatic cysts The various abnormalities which fall under this description can affect the absorption of fats directly, by decreasing the secretion of lipase, and sometimes also indirectly, by the pressure of the enlarged pancreas against the bile duct As might be expected cystic fibrosis of the pancreas has been shown to cause decreased absorption of vitamin A [39, 46]

Giardiasis According to Katsampes *et al* [47] the absorption of vitamin A, and also of fat, is defective in children infected with the parasite *Giardia lamblia*

Pneumonia Lindqvist [16] made serial estimations of vitamin A, carotenoids and cholesterol in the blood and of vitamin A in the urine, in specimens collected from 71 patients with lobar pneumonia and from 25 patients with broncho pneumonia The results obtained for a typical case of lobar pneumonia are shown in Fig 30 (The values for vitamin A have been corrected by a factor of 0 46, to allow for the suspected error in calibration) It will be seen that during the active stage of the disease, when the body temperature must have been high vitamin A was low in the blood and was excreted in large amounts in the urine During recovery

the vitamin disappeared rapidly from the urine, and at the same time the level in the blood started to increase The curve for the blood cholesterol followed the same tendencies as for vitamin A but the differences between the readings during the active and recuperative periods were less wide The carotenoid levels were low during the active phase, and remained low during recovery

It is worth emphasis that the first four readings for vitamin A in the case under discussion fell under 40 i u the level below which defective dark adaptation is to be expected[48] The final reading at 148 i u, was above the average of 106 i u, which was found by Lindqvist for a group of 25 healthy adults, mostly males Very low levels of carotenoids were also found in the pneumonia patient during the active stage of the disease but there was no dramatic increase during recovery to parallel that shown by vitamin A Even in his group of healthy adults however Lindqvist found an average of only 49 μg for carotenoids, which was much lower than the average of 137 μg calculated in Chapter 29 for subjects of different nationalities, mainly British and American

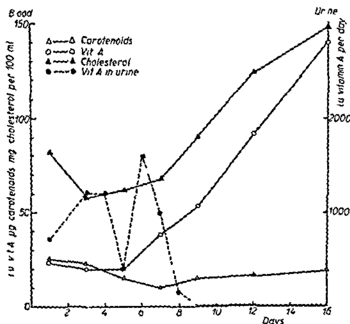


Fig 30 Pneumonia Changes in the levels of vitamin A carotenoids and cholesterol in the blood and in the excretion of vitamin A in the urine at various stages in a typical case (Lindqvist 1938)

Another extensive investigation on the effect of pneumonia on the vitamin A status was made on infants and children by Josephs[49] Previous work was reviewed not only on the decrease of vitamin A in pneumonia[14 50–52] but also on the effect of infections in lowering the level of lipids in the

References p 440

blood[53, 54] It seemed important, therefore, to find out how far the changes in vitamin A were merely a reflection of those in lipids As in Lindqvist's work the results for each patient were presented separately, without any attempt to find averages according to the stage of the disease, or some other ordinate Complications were also introduced by the necessity of dividing patients according to age groups, and by further dividing them according to whether they were dosed with vitamin A or carotene

In view of the wealth of data at Joseph's disposal, however, it seems safe to accept his considered conclusions The changes in vitamin A, carotenoids and lipids in his experience, followed the same tendencies, but did not run completely parallel in individual cases Of the three variables vitamin A was *most rapidly reduced during the active stage of the disease* In children over 2 years old, but not in young infants, the phase of recovery was characterised by a temporary elevation of both vitamin A and lipids above the normal average *The peaks of this increase, however, did not necessarily coincide* for these two variables It was concluded that both vitamin A and lipids in the blood are probably subject to the same influences, but that their responses are different *both in speed and in intensity*

The vitamin A reserves in the livers of 22 patients who had died from pneumonia were found by the author[2] to be low, with a median of only 60 i u per g *The ranges of reserves found by Wolff*[1] *and Fox*[5], *however,* were only slightly below those in accidental death It should be borne in mind that these observations were made before modern methods for the treatment of the disease *had become available*

Rheumatic fever The metabolism of vitamin A in rheumatic fever has been carefully investigated in view of strong suspicions that a defective diet may be a *conditioning factor*[55, 56] Coburn and his colleagues[57] found that vitamin A and carotene were much reduced in the blood plasma of rheumatic children Jacobs, Leitner, Moore and Sharman[58] confirmed this finding *for the acute phases of the disease*, and carried out numerous serial examinations in order to study the relationships between vitamin A temperature, the erythrocyte sedimentation rate, and the time since the onset of the disease *For 100 cases of acute rheumatism* their results, arranged according to ranges of body temperature, were as follows

Temperature	*No of estimations*	*Carotenoids μg/100 ml*	*Vitamin A i u /100 ml*	*E S R mm/hr*
Below 98 0° F	170	63	105	17 7
98 0–98 8	381	62	104	22 6
98 9 100	44	68	74	53 5
Over 100	28	43	50	83 6

It will be seen that vitamin A fell, and the erythrocyte sedimentation rate increased, when the body temperature rose over 98 8° F The level of carotenoids was less sensitive to pyrexia, and a fall in the average value was only observed when the temperature exceeded 100° F In agreement with previous observations by other workers the same relationship between vitamin A and body temperature was found in other diseases including pneumonia and acute tonsillitis

The changes in the average for the plasma vitamin A and for the other variables, according to the time since the onset of the disease are summarised graphically in Fig. 31 It will be noticed that during recovery from the acute phase of the disease the body temperature was first to regain its normal level, followed by vitamin A and carotenoids and finally by the E S R Jacobs and his colleagues pointed out, however that the inverse relationship between temperature and vitamin A did not always hold good in individual cases Similar inconsistencies were also found in other diseases and instances were given of very low values for vitamin A which they had observed in patients, without pyrexia, who had died 5–25 days later It is well known, of course, that in rheumatoid arthritis high E S R values may be associated with normal body temperature

In rheumatic children attacks of acute fever tend to recur, and the disease may progress with the development of endocarditis There seems danger, therefore, of a more serious strain on the vitamin A reserves than is imposed by a single attack of acute infection, as in pneumonia Jacobs and his colleagues recalled that Ellison and Moore[8] had reported an average vitamin

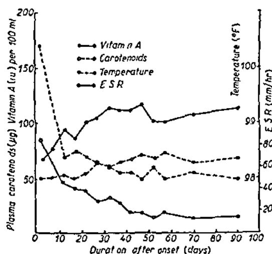


Fig 31 Rheumatic fever Changes in the average levels of plasma vitamin A carotenoids body temperature and erythrocyte sedimentation rate found in repeated observations on 100 patients at various times during recovery from an acute attack of rheumatic fever (Jacobs Leitner, Moore and Sharman, 1954)

References p 440.

A reserve of only 28 i u per g of liver in nine children who had died from heart disease, as compared with 129 i u in 12 children who had died by accident The possibility of the vitamin A status reaching so low a level as to complicate the original disease process, by an additional strain, cannot therefore be overlooked We must not forget, however, that nutrients other than vitamin A may also be affected Thus Abbasy, Gray Hill and Harris [59] found that the vitamin C status, as indicated by the extent of urinary excretion, is depressed in acute rheumatic fever

Infective hepatitis Acute hepatitis could hardly be expected to leave the vitamin A status unaffected, since both pyrexia and a hepatic reaction are involved In early experiments by Popper, Steigmann and Zevin [60] the plasma levels of vitamin A in toxic hepatitis were found to be very low After massive test doses had been given the increases observed in the blood were usually less than one third of those found in normal subjects

Detailed studies on 30 patients with infective hepatitis, and two with jaundice caused by arsenotherapy, were made by Harris and Moore [61] The low levels of vitamin A in the early stages of the disease were confirmed, with an average of only 55 i u per 100 ml during the first days after admission to hospital as compared to 124 i u after 20–29 days An inverse relationship was again established between body temperature and the blood vitamin A During recovery vitamin A increased into the normal range, and the intensity of jaundice, as indicated by bilirubin estimations, decreased (Fig 32) The repair of liver damage during the same period was indicated by an increased formation of hippuric acid after the ingestion of sodium benzoate, and by a return to normal of the reduced prothrombin index Changes in carotenoids tended to run parallel with those for vitamin A In absorption tests, in which single massive doses of vitamin A were given the increases in the plasma levels observed in the patients with hepatitis were always lower than in normal subjects In several instances increases of only 30–60 i u per 100 ml were observed, as compared with 200–800 i u in normal subjects In the limited number of cases studied in this way, however, no consistent relationship could be traced between the levels in the plasma before and after dosing or between the extent of the increase in the plasma and the stage of the illness Vitamin A was sometimes excreted in the faeces of the patients with hepatitis in amounts up to half the dose ingested, but no vitamin was found after dosing in the faeces of control subjects In a few instances small amounts of vitamin were detected in the urine of the patients with hepatitis

Estimations of vitamin A in the livers of three patients, outside the main investigation who had died from hepatitis indicated clearly that the low levels of vitamin A in the plasma are not caused by a lack of vitamin A

in the liver Thus in one patient the level in the plasma was only 19 i u per 100 ml shortly before death although a very high liver reserve of 900 i u per g was found at autopsy

Harris and Moore explained the inconsistencies found between different patients on the assumption that the absorption and metabolism of vitamin A may be disorganised at several different points Thus there may be failure in absorption by the intestines failure in absorption by the liver failure in the release of pre existing hepatic stores into the blood stream and probably also an abnormal rate of metabolism of the vitamin A patient who has a normal level of vitamin A in the blood plasma before dosing but who fails to show a normal response after dosing may have preserved the power of mobilising the vitamin from the liver but lost the power of absorption from the intestines

In contrast a patient with a low resting level of vitamin A but with a fairly good response to dosing may have lost the power of absorption and mobilisation of the vitamin by the liver but preserved the power of ab sorption from the intestine It seems possible indeed that relatively poor absorption from the intestines may be made to appear normal if the inter change of vitamin between the blood and liver is seriously disorganised Thus small amounts of vitamin absorbed into the blood stream will be allowed to accumulate instead of being absorbed by the liver Irregular responses to massive dosing were also observed in catarrhal jaundice by Breese and McCoord[62].

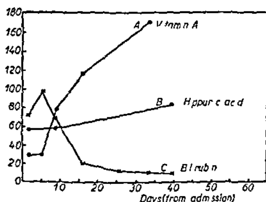


Fig 32 Infective hepatitis Changes in plasma vitamin A serum bilirubin and hippuric acid synthesis at various times after admission of the patient to hospital (Harris and Moore 1947) Curve A = plasma vitamin A (i u per 100 ml) Curve B = hippuric acid synthesis (g of sodium benzoate detoxicated in first hour × 100) Curve C = serum bilirubin (mg per 100 ml × 10)

Other infections Studies of the vitamin A status have also been made in many other forms of infection although the data accumulated have generally been less extensive than for the infections which have

already been mentioned Thus Spector McKhann and Meserve [44] observed low resting levels for vitamin A in patients with measles typhoid and scarlet fever They also reported that in these conditions the level of vitamin A in the plasma failed to show its usual response to dosing In six cases of chronic infection, including pansinusitis bronchiectasis, osteomyelitis and tuberculous meningitis, the level in the blood failed to show a normal rise after dosing, with no increase at all in three instances An 11 months old infant suffering from post nasal discharge, due to infected adenoids, failed to grow and showed poor absorption of vitamin A Growth was restored, and the absorption of vitamin A improved, by adenoidectomy

Breese, Watkins and McCoord [63] found that in 29 patients with severe pulmonary tuberculosis the increases of vitamin A observed in the blood after massive dosing, averaged only half those observed in 25 normal subjects There were indications that absorption was lower in patients with severe than with slight intestinal symptoms Huet [64] found low resting levels of vitamin A in the blood of tuberculous patients Early work by Heymann [65] had shown that in various infections, including pneumonia, influenza and sepsis, the absorption of carotene by infants was greatly reduced The disturbance in absorption persisted for two weeks after the temperature had returned to normal

In his surveys of liver reserves the author [2] found low ranges in infections of the head and spine, with a median of 73 i u per g and also in pyogenic *infections in various other parts of the body, with a median of* 51 i u For 13 cases of kidney and bladder infections a median of only 19 i u was found In seven *cases with renal calculi, usually complicated by infections* the reserves ranged from 9 to 650 i u per g with a median of 86 i u [66] We may remind *ourselves that the median for accidental death was* 220 i u per g

Cirrhosis of the liver Cirrhosis involves the replacement *of many of the* liver cells by fibrous tissue, which presumably has little affinity for vitamin A Serious derangement *in the absorption and* storage of the vitamin are therefore inevitable Wolff [1] first reported that the liver reserves in cirrhosis are usually low, and often *completely absent* Similar findings were reported later by Aschoff [7] and Woo and Chu [11] In a total of nine cases Moore [2] *found* reserves of 0, 0, 3 6, 6 *and* 6 i u in six of the cases but higher reserves of 90, 375 and 525 i u in the remaining three cases Since cirrhosis can take several forms, and can arise from various causes, it is perhaps not surprising that the vitamin A status should deteriorate in some cases, but should appear to *remain unaffected in others*

Evidence from other investigators has indicated clearly that cirrhosis also causes a defect in the physiological action of vitamin A as indicated

by hemeralopia Thus Patek and Haig [67] found that dark adaptation was defective in 19 out of 24 patients with cirrhosis, and that the abnormality persisted even when a diet rich in vitamin A was given Wohl and Feldman [68] found that dark adaptation was abnormal in 9 out of 10 alcohol addicts, whose livers were presumably cirrhotic. In contrast adaptation was normal in three patients with hepato-cellular jaundice, but without permanent damage to the liver.

The level of vitamin A in the blood plasma was examined by Ralli Bauman and Roberts [69] For five patients with alcoholic cirrhosis of the liver the average resting value was only 44 i u per 100 ml, which was increased to 141 i u at three hours after a dose of 100,000 i u of the vitamin. In contrast the resting level for normal subjects was 168 i u, which was increased to 374 i u. after dosing Haig and Patek [70] studied dark adaptation times, and the levels of vitamin A and carotenoids, in 49 patients with Laennec's cirrhosis, which is the most common form as induced by alcoholism Observations were also made on patients with non-hepatic diseases, and on normal subjects. The following average values were found·

	No of cases	Dark adaptation time (mins)	Blood plasma Vitamin A i u/100 ml	Carotenoids µg/100 ml
Cirrhosis, uncompensated	26	19 7	65	72
Cirrhosis, compensated	23	17 3	122	88
Non hepatic	38	15 2	154	121
No disease	44	13 1	198	144

From these findings it was concluded that the most informative index for prognostic purposes was vitamin A, since the average for compensated cirrhotic patients was nearly double that found for uncompensated patients

Chronic nephritis In this disease lower ranges of liver reserves have been observed than in any other abnormality, except iver cirrhosis, for which an adequate amount of data has been collected. For 48 nephritic cases, aged 15–59, the author reported a median of only 25 i u per g, as compared with 220 i u for 40 cases, within the same age range, who had died within 7 days of an accident (Fig 33). Low ranges, or extremely low values for individual cases, have also been reported by several other investigators [3, 6, 7, 11, 12]. Wolff [1] found a low average for the vitamin A reserves of a large group of unclassified cases of kidney disease, mostly chronic nephritis

Without further evidence of a disturbance in vitamin A metabolism the reduction in the liver reserves might perhaps have been ascribed to the prolonged period of decline in chronic nephritis, associated with an inadequate intake of food Subsequent observations that nephritic patients usually

References p 440

excrete vitamin A in their urine, however, have suggested that the relationship between the disease and the vitamin must be more direct [19, 20, 21, 28, 71] The question whether long sustained urinary excretion in itself can adequately explain the depleted liver reserves obviously deserves consideration

At death the loss of vitamin A from the liver of a typical case of chronic nephritis may be estimated as about 300,000 i u which may be compared with a daily requirement of 2,500 i u From the data of Lawrie Moore and Rajagopal [32] the urinary losses in chronic nephritis cannot have exceeded about 500 i u daily The excretion of the vitamin appeared to be of interest therefore as a sign of disorganised metabolism of the vitamin and not as a full explanation of the depletion Daily losses of more than 1 000 i u, however, were later reported by Johns, Hoch and Marrack [72] They noticed that the liver reserves were low in cases with a long history of renal failure but normal in cases with a short history In their opinion the depletion after prolonged nephritis could be adequately explained by the constant urinary excretion

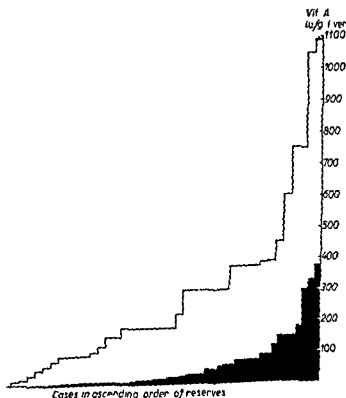


Fig 33 Chronic nephritis The liver reserves in 48 cases of the disease aged 15–59 years are shown in the lower black skiagram They may be compared with the reserves in 40 cases of accidental death which are shown in the upper white skiagram In each group columns representing individual reserves have been arranged in ascending order (see also Fig 23 p 358) The sides of the columns are not shown To compensate for the different numbers of cases slightly wider columns have been drawn for accidental death than for nephritis

It may seem surprising that the level of vitamin A in the blood in chronic nephritis is increased rather than reduced [16, 21, 72] Presumably the power of the liver to hold the vitamin is decreased or the carrying power of the blood is increased Marrack and his colleagues found that in blood most of the increase in vitamin A above the normal level was due to the free alcohol In the urine the vitamin was present both as the free alcohol and as esters with the proportion of esters greater than in blood serum collected at the same time In a specimen of nephritic urine which was recently examined by Sharman [73] however the vitamin was present predominantly as the free alcohol

Lawrie Moore and Rajagopal [30] could detect small amounts of vitamin A in the kidneys of only 3 out of 13 cases of chronic nephritis In contrast the vitamin was present in the kidneys in 14 out of 15 cases of accidental death Another point worth mentioning is that during the author s studies on the liver reserves of vitamin A in various diseases it was repeatedly noticed in cases of chronic nephritis that the intensity of the yellow colour of the ether extract was much above the average

Nephrosis In this condition oedema is associated with a pronounced fall in the plasma proteins and an ever greater rise in the lipids particularly cholesterol It is only to be expected therefore that the vitamin A status must be affected

Boller Brunner and Brodaty [20] reported that vitamin A is excreted in the urine in nephrosis and this finding has been confirmed [16, 32, 74] Bonfils and Marnay [74] noticed that in a patient with nephrosis the increase in vitamin A observed after a massive dose was abnormally large Kagan *et al* [75] not only confirmed this finding in nephrotic children but also found high resting levels of vitamin A in the blood of the same subjects Later Kagan and Kaiser [76] reported that the liver reserves of vitamin A at autopsy were also high in nephrosis In three cases values of no less than 2 000 i u 2 300 i u and 6 000 i u per g were observed These findings seemed to exclude the possibility that the high resting levels observed in the plasma were maintained at the expense of the liver reserves It is possible however that the picture may have been complicated by the administration of massive doses of the vitamin The child who had the lowest level of vitamin A in the liver was said to have received no added sources of vitamin A for two years before her death Mention is made nevertheless of the administration of test doses

Prostate hypertrophy Moore [2] found a low median of only 40 i u per g in the livers of a group of 23 old men who had suffered from prostate diseases Schneider and Widmann [77] failed to detect vitamin A in the blood serum of 4 out of 5 cases of prostate hypertrophy These findings seem worth mentioning in view of the histological structure

References p 440

of the prostate which suggests that it should be vulnerable to the metaplastic changes which are one of the characteristic effects of vitamin A deficiency Wolff[1], however, found a high average for the liver reserves in 8 cases of prostate hypertrophy

Diabetes Wolff [1] and Moore [2] both found ranges of liver reserves of vitamin A in diabetes which were much above those in accidental death Breusch and Scalabrino [6] also commented on a very high value found in a single case

Another early finding, dating even from before the discovery of vitamin A, was the frequent occurrence in diabetes of yellow pigmentation of the skin This "hypercarotenosis" will be described more fully in our next chapter, but for the present it is sufficient to note that the skin abnormality is accompanied by an unusually high level of carotenoids in the blood plasma

Apparently without knowledge of the evidence of high liver reserves of vitamin A, Ralli, Brandaleone and Mandelbaum [78] concluded that diabetes causes a defect in the conversion of carotene In support of this view they found that when diabetic patients were given large doses of carotene either in oily solution or as carrots the increase of the provitamin in the blood was greater, and more rapid, than in normal subjects Clausen and McCoord [60] confirmed that the blood carotenoids were often high in diabetes and Brazer and Curtis [79] found that diabetic patients had defective dark adaptation

Murrill *et al* [80] agreed with previous workers about the high levels of carotenoids in the blood of diabetics Thus for diabetic subjects of both sexes they found an average of 291 μg per 100 ml, as compared with 206 μg for normal subjects The levels of vitamin A in diseased and normal subjects however, were virtually identical, averaging 103 and 106 i u respectively No difference could be observed, moreover, between the rates of disappearance of carotene from the blood of diabetic and normal subjects after massive dosing with the provitamin

Kimble Germek and Sevringhaus [81] estimated vitamin A and carotenoids in the blood of 116 diabetic patients Their results not only failed to support the theory of a defective conversion of carotene to vitamin A but even cast doubts on the significance of previous observations of high carotenoids in diabetic blood plasma Thus when high levels for carotenoids were found they were usually associated with high levels of vitamin A The most frequent abnormality, however, was for both carotenoids and vitamin A to be unusually low To a great extent the levels of vitamin A appeared to be influenced by the nature of the lesions accompanying the diabetes Thus fever and coma were associated with low values for vitamin A, but nephritis was associated with high values for both vitamin A and carotenoids Hillman

and Nerb [80] again confirmed that high blood carotenoids did not imply that the conversion to vitamin A was defective In 65 patients they could establish no correlation between hypercarotenaemia and the degree of damage to the liver

Kimble and her colleagues [81] suggested that the ability to convert carotene might depend on an adequacy of protein in the diet The patients found to have hypercarotenaemia by early workers were presumably subsisting on diets low in protein whereas her own patients were receiving an adequate diet Even greater importance however may be attached to the inclusion of large amounts of green vegetables and carrots in the diets of diabetic patients as substitutes for carbohydrates With the increased use of insulin there has been less need to resort to restriction of the carbohydrate intake and a lesser use of vegetables may account for the difference between early and later observations on the incidence of hypercarotenaemia The high liver reserves of vitamin A found in diabetes by early workers could also be explained by a high intake of carotene

There may be some temptation therefore to regard the problem of vitamin A metabolism in diabetes as having been fully solved A suspicion may remain however that the metabolism of both carotene and vitamin A may be depressed If the expenditure of vitamin A were even more retarded than the conversion of carotene it is clear that increased levels of both substances could result

Thyroid diseases Abnormalities in the metabolism of vitamin A and carotene have been reported in diseases involving both deficiency and excess of vitamin A In support of this view Wohl and Feldman [83] found that dark adaptation was abnormal in both types of abnormality

In hypothyroidism the frequent occurrence of hypercarotenaemia has long been recognised [50, 84, 87] Josephs [87] made a careful study on the blood serum in 25 cases of cretinism or juvenile myxoedema Increases in lipids and cholesterol accompanied the increases in carotenoids but were less pronounced Moreover the carotenoids responded less rapidly to treatment with thyroxine than did the lipids or cholesterol As in the investigation of diabetes a defect in the conversion of carotene to vitamin A was suspected

In regard to hyperthyroidism in human patients very little information seems available For liver specimens from small numbers of patients who had died of exophthalmic goitre both Wolff [1] and Moore [2] found ranges of vitamin A reserves which exceeded those found in accidental death

Claims that patients with either the simple goitre of adolescence [88] or exophthalmic goitre [89] benefit from treatment with vitamin A have not been

References p 440

TABLE 52

THE EFFECT OF VARIOUS DISEASES ON THE VITAMIN A STATUS[a]

Disease	Vitamin A					Defective dark vision
	Decreased intestinal absorption	*Decreased level in blood*	*Decreased stores in liver*	*Excretion in faeces*	*Excretion in urine*	
Coeliac disease	++	+		+		
Sprue	++	+		+		
Obstructive jaundice	++	+		+	+	
Pancreatic cysts	++	+		+		
Pneumonia	+	++	+		+++	
Rheumatic fever	+	++	++			
Infective hepatitis	++	++	normal		+	+
Chronic infections	+	+	++		+	
Cirrhosis of liver	++	++	+++		+	+
Chronic nephritis		Increased ?	+++		++	
Nephrosis	Increased ?	Increased	Increased ?		++	
Diabetes		Normal	Increased		+	
Hypothyroidism		Increased ?	Increased ?			+
Hyperthyroidism			Increased ?			+

[a] The different numbers of crosses are intended to indicate the intensity of the abnormality In regard to the excretion of vitamin A in the urine one cross has been given on the basis of a report that excretion sometimes occurs in the disease in question but it must not necessarily be concluded that the excretion is a regular feature in the disease Where spaces are left blank no information is available

confirmed Complications arose through the presence of iodine in some of the vitamin A concentrates which were administered

Experimental studies on relationships between vitamin A and thyroxine are discussed in Chapter 34

Intestinal diseases Wolff[1] found the extremely low average of only 7 I U per g for the liver reserves of vitamin A in three cases of enteric fever Nicholls[10] also reported four cases of enteritis in Ceylonese children in which no vitamin A at all could be detected in the liver In gastric and duodenal ulceration the liver reserves of vitamin found by Wolff[1] were unusually high while Moore[2] found reserves of the order to be expected in most chronic diseases Further studies on the vitamin A status in enteritis seem desirable

Conclusions The systematisation of our knowledge on the vitamin A status in common diseases is made difficult by the large gaps in the information which is available Few investigators have studied even a single disease from all possible angles and it is often necessary to compare results that have been obtained by different workers by different methods and at widely different times As an example we may remember that the studies on the liver reserves of vitamin A in diabetes were made many years earlier than those on levels of carotenoids and vitamin A in the blood plasma Attempts to combine the two sets of data may be complicated therefore by changes in the treatment of diabetes which have taken place during the interval

In spite of such difficulties however it will be seen from the summary in Table 52 that substantial progress has already been made towards a full understanding of the interrelationships between vitamin A and disease Thus it has been amply demonstrated that the influence of disease is not general and non specific but is varied according to the nature of the lesions involved While some diseases cause low levels of vitamin A in the blood others cause high levels In some diseases the liver reserves of vitamin A are low and in others high without any consistent correlation between the levels in the liver and blood Many fascinating problems remain for careful investigation with the aid of modern methods for the separation and estimation of vitamin A and the carotenoids It seems safe to prophesy that the information so obtained will be of value not only in guiding dietitians but also in informing pathologists on certain aspects of the biochemical changes which underlie disease

TABLE 52

THE EFFECT OF VARIOUS DISEASES ON THE VITAMIN A STATUS[a]

Disease	Vitamin A: *Decreased intestinal absorption*	*Decreased level in blood*	*Decreased stores in liver*	*Excretion in faeces*	*Excretion in urine*	*Defective dark vision*
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Sprue	++	+		+		
Obstructive jaundice	++	+		+	+	
Pancreatic cysts	++	+		+		
Pneumonia	+	++	+		+++	
Rheumatic fever	+	++	++			
Infective hepatitis	++	++	normal		+	+
Chronic infections	+	+	++		+	
Cirrhosis of liver	++	++	+++		+	+
Chronic nephritis		Increased ?	+++		++	
Nephrosis	Increased ?	Increased	Increased ?		++	
Diabetes		Normal	Increased		+	
Hypothyroidism		Increased ?	Increased ?			+
Hyperthyroidism			Increased ?			+

[a] The different numbers of crosses are intended to indicate the intensity of the abnormality In regard to the excretion of vitamin A in the urine one cross has been given on the basis of a report that excretion sometimes occurs in the disease in question, but it must not necessarily be concluded that the excretion is a regular feature in the disease Where spaces are left blank no information is available

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CHAPTER 33

Hypercarotenosis and Hypervitaminosis A in Humans

Abnormalities in human subjects may result from the consumption of grossly excessive amounts of either preformed vitamin A or its provitamins [illegible] by an excessive [illegible] d usually readily curable Hypercarotenosis may indicate nothing more than an unusually voracious appetite for carrots, but may sometimes be a secondary sign of disease Although the two abnormalities are very different in many ways both may conveniently be discussed in the same chapter as being effects of excess

HYPERCAROTENOSIS

Pigmentation of the skin Most authors credit von Noorden [1] with giving in 1907 the first description of the yellow dis coloration of the skin which results from a grossly excessive intake of carotene, or sometimes from defects in its metabolism He observed the pigmentation in patients with diabetes, who often ate large amounts of vegetables in efforts to restrict their consumption of carbohydrates Soon afterwards Moro [2] noticed that normal infants developed similar discoloration when they were given carrots The yellowness, according to all accounts, was always most intense on the palms of the hand and soles of the feet In 1924 Parkes Weber [3] described its appearance in a diabetic patient as "a curious canary yellow coloration of the skin' He pointed out that the condition could be distinguished from jaundice by the sclerotics remaining normal

In view of the appearance on the skin of the outward signs of the excess of carotene the description "xanthosis cutis", which was used by Parkes Weber, seemed quite appropriate The term "hyperlipochromia', used by Stannus [4], also appears reasonable Most recent workers, however, have preferred the description "carotenaemia", which is perhaps less suitable

The blood plasma normally contains carotenoids and the margin between the highest levels found in patients with normal skin and in those with discoloured skin is not wide "Hypercarotenaemia" would be a more adequate description

Excess of carrots Abnormalities in carotenoid metabolism which are associated with diseases such as diabetes and hypothyroidism are discussed more fully elsewhere (Chaps 32 and 37) In the present chapter however we have to deal with the occurrence of hypercarotenosis in otherwise healthy subjects Undoubtedly its most frequent cause is a grossly excessive consumption of carrots

In 1919 Hess and Myers [5] confirmed that the condition was not necessarily associated with diabetes by observing its appearance in two out of 25 children who were given large amounts of carrots In the same year Schussler [6] reported three cases in adults living on diets largely composed of carrots

In Britain the cultivation and consumption of carrots was encouraged during the second World War by intensive propaganda Almond and Logan [7] gave an interesting and amusing account of instances in which the official advice was accepted with excessive enthusiasm In subjects who ate 2 kg or more, of carrots each week pigmentation was developed in the usual sites and was attributed by unsuspecting practitioners to jaundice pernicious anaemia haemolytic anaemia or cholelithiasis One housewife who kept munching carrots during pregnancy not only became yellow herself but transferred the pigment to her baby Almond and Logan were guided to the true explanation of the abnormalities by the absence of pigmentation of the sclerotics When the consumption of carrots was reduced to an ordinary level the pigmentation invariably disappeared within a few weeks

McConaghey [8] observed pigmentation in two women who had several points in common Both were rather plump women approaching the menopause who complained of lethargy and nervousness They both had slight hypochromic anaemia and an insatiable desire for carrots One was induced to moderate her appetite and lost her yellow colour within a few weeks The other on being assured that her condition was not serious preferred to remain yellow rather than deny her craving A further instance of a mistaken preliminary diagnosis was reported by Auckland [9] Yellow pigmentation of the palms soles face and soft palate was seen in a woman who was admitted to hospital with threatened abortion She had worked in a munition factory and an accumulation of dye was suspected It was found out later however that she had been eating 3 kg of carrots weekly for about a year The colour disappeared within 4 weeks of the omission of carrots from her diet

References p 454

Other sources of pigmentation

Although excess of carrots is the most common cause of pigmentation in healthy subjects other vegetables have also been implicated Miyake [10] described "aurantiasis" in a Japanese woman who ate 1200 oranges in less than six weeks Hashimoto [11] reported pigmentation as the result of eating squashes Josephs [12] observed instances of pigmentation which were caused by oranges in an infant and by spinach in a young adult

Hypercarotenaemia

In 1914 Van den Bergh and Snapper [13] demonstrated the presence of lipochrome pigments, presumably in excessive amounts, in the blood plasma of diabetic patients whose skin was coloured yellow Buerger and Reinhart [14] [15] confirmed that the intensity of pigmentation in the skin ran parallel with the level of liprochrome in the blood Josephs [12] found that three out of four cases with skin pigmentation had levels of about 600 μg of carotene per 100 ml of serum, as compared with a normal average of about 150 μg McConaghey [8] confirmed in his cases that the carotene contents of the serum were more than twice the normal average

Excessive carotene and health

As already explained the appearance of yellow pigmentation in human skin may in some cases indicate serious disease, but in others may merely reflect an unusually high consumption of carrots, or of some other vegetable rich in carotenoids

In many instances, however, the significance of the hypercarotenosis may lie between these two clear extremes Thus Josephs [12] has pointed out that diets containing enough carotene to cause yellow pigmentation may often be deficient in calories The patient may therefore be suffering from general malnutrition Josephs concluded, moreover, that excess of carrots may in itself be harmful even if the diet is otherwise adequate A man of 36 who had eaten large amounts of carrots for many years lost his yellow pigmentation, and gained in weight, without any dietary change other than the omission of the carrots

HYPERVITAMINOSIS A

Causes of excess

requirement for

cannot be incre

human hypervitaminosis are rare the cases described have been ...

authenticated

The most important cause of the condition is the injudicious administra

tion, usually to children, of massive doses of vitamin A concentrates When the dosing is prolonged abnormalities in the structure of the bones are the most characteristic lesion In this respect the lesions are of the same type as those seen in the hypervitaminotic rat When only a single, very massive dose has been given the main effect seems to be an excessive production of cerebro-spinal fluid, which in infants is made manifest by a transient hydrocephalus

A second cause of hypervitaminosis is the consumption of polar-bear liver, which is a very rich source of vitamin A (Chap 13) As might be guessed only Arctic explorers are affected and the main abnormalities are distress, nausea and pealing of the skin The first two of these symptoms might well be caused by a sudden rise of the cerebro-spinal fluid pressure, following a single massive intake of the vitamin

Prolonged excessive dosing In 1944 Josephs gave the first account of hypervitaminosis A in a human child A three-year old boy, who had received halibut-liver oil equivalent to about 240,000 i u of vitamin A daily since he was three months old, became seriously ill The condition was characterised by enlargement of the liver and spleen, hypoplastic anaemia leucopenia precocious skeletal development, clubbing of the fingers and coarse sparse hair Serum lipoids reached 1140 mg per cent, as compared with a normal range of 550–750 mg Serum phosphatase was high and serum protein low especially the globulin fraction The appetite for halibut liver oil was abnormal and the level of vitamin A in the plasma was over 900 i u per 100 ml

When the excessive dosing was stopped the vitamin A in the plasma fell to 650 i u after 20 days, 480 i u after 3 months 280 i u after 7 months, 40 i u after 2½ years Most of the symptoms cleared up promptly, but the enlargement of the liver and spleen, and the abnormal bone growth, persisted Josephs pointed out that other children who were given massive doses of vitamin A suffered no ill effects In these children there was no increase in the level of vitamin A in the plasma except for the usually temporary rise soon after dosing

Thus an eight month's old infant which had been given 500,000 i u of vitamin A daily showed no ill effects, and had a plasma level of only 110 i u per 100 ml, with lipoids only 750 mg Two infants given 200 000 i u daily for several weeks showed only slight increases in the vitamin A contents of their serum, and values considered normal were found after dosing had been stopped for one week Josephs suggested that the capacity of the hypervitaminotic subject to dispose of vitamin A must be defective, possibly through injury to the reticulo-endothelial system caused by the prolonged overdosing.

References p 454

The resemblance to scurvy The next case of hypervitaminosis A was described by Toomey and Morisette [16], and was particularly interesting in showing at least a superficial resemblance to scurvy It will be recalled that workers with rats had already commented on this similarity (Chap 28) On admittance to hospital a 23 month old male child was found to be suffering from soreness and swelling of the arms and legs The skin on the face was erythematous, the lips dry and cracked, and the hair sparse The liver and certain lymph nodes were swollen, but not the spleen The child was irritable and had a poor appetite In X ray examination broad growth lines were found at the ends of the long bones Other abnormalities were stippling of the distal femoral epiphyses, a periosteal line along the lateral aspects of the right femur and left tibia, and pronounced periosteal thickening on the posterior and medial aspects of the ulnar bones

The soreness of the limbs and the anorexia, but not the skin abnormalities, were relieved after a short stay in hospital The case was diagnosed as healing scurvy, and the patient was discharged, only to be readmitted three weeks later with a recurrence of the symptoms On questioning the mother it was found that she had habitually given her child unreasonably large doses of vitamins, including 250 000–500,000 i u daily of vitamin A as percomorph oil The plasma vitamin A was 800 i u per 100 ml with raised phosphatase In subsequent treatment the production or cure of the symptoms could be effected rapidly by giving or withholding large doses of vitamin A A concentrate of distilled vitamin A esters, which was virtually devoid of vitamin D, readily produced the symptoms When the excessive dosing was finally stopped it took 6 months for the plasma vitamin A to fall within the normal range, while blood phosphatase was abnormally high 2½ years after the first illness The child had made a complete clinical recovery, however, within 20 months There was no evidence of permanent damage to the liver

Other recorded cases in children Two further cases of hypervitaminosis A in young children were reported by Rothman and Leon [17] and by Dickey and Bradley [18], seven by Caffey [19] two by Fried and Grand [20] and one by Wyatt, Carabello and Fletcher [21] Gribetz, Silverman and Sobel [22] reported two cases of their own and gave an excellent summary of previous observations In all of 16 recorded cases they pointed out, the patients had always been over 6 months old Cortical thickening of the bones was an invariable feature (Plate 36) Painful swellings in the extremities were found in 13 cases, irritability in 12 and pruritis in 10 In all the 14 cases examined for the levels of Ca and P in the blood normal values were found Hepatomegaly was found in only 7

cases failure to stand in 6 sparse coarse hair in 5 fissuring of the lips in 4 and failure to gain weight in 3 cases

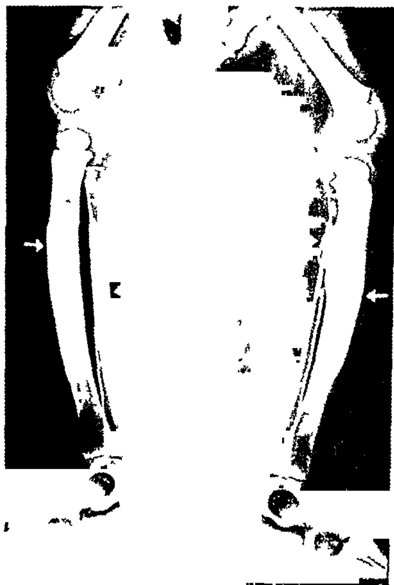


Plate 36 Hypervitaminosis A in a 1 months old girl who had been receiving 240 000 I U of vitamin A daily as indicated by X ray examination of the legs Note the external cortical thickening of both tibia and of the right fibula (Gribetz Silverman and Sobel 1951)

In spite of the resemblance between the symptoms of hypervitaminosis A and those of scurvy no evidence of vitamin C subnormality was found Thus when Gribetz and his colleagues gave massive doses of ascorbic acid to a 2½ year old boy with hypervitaminosis the urinary excretion of the excess of vitamin indicated prompt saturation There was likewise no evidence of

References p 454



Plate 37 Hypervitaminosis A in a woman caused by the consumption of 500 000 i u of vitamin A daily for 8 years Note the stooping posture which was associated with pain in movement and also the bulge in the right temporal area (Gerber Raab and Sobel 1954)

a secondary deficiency of vitamin K such as occurs in the hypervitaminotic rat The prothrombin time for the same boy was 14 1 seconds which differed but little from 12 1 seconds found in a control experiment

Since it was suspected that inefficient functioning of the liver might be a predisposing factor in the development of hypervitaminosis loading tests were made with massive doses of the vitamin In spite of the higher level before dosing a much greater increase was found after dosing in the hyper vitaminotic boy than in a normal child In routine estimation of vitamin A in the plasma, levels of 2000–3000 i u per 100 ml were observed It is particularly interesting that as much as 70 per cent of this very high concentration was in the form of the free alcohol Possibly the presence of high ... than of its esters

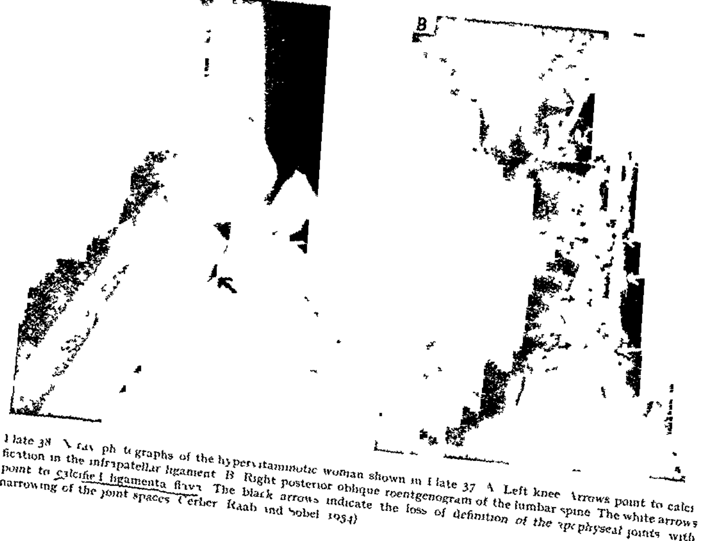


Plate 38 X-ray photographs of the hypervitaminotic woman shown in Plate 37 A Left knee Arrows point to calcification in the infrapatellar ligament B Right posterior oblique roentgenogram of the lumbar spine The white arrows point to calcified ligamenta flava The black arrows indicate the loss of definition of the apophyseal joints with narrowing of the joint spaces (Gerber Raab and Sobel 1954)

Hypervitaminosis A in adults Detailed studies on an interesting case of hypervitaminosis A in an adult have been described by Gerber, Raab and Sobel [23] A 28 year old woman came under their care with a history of eight years of persistent illness, for which she had received treatment on ten occasions in various hospitals She suffered from headaches, a coarse and itching skin blurring of vision and diplopia and stiffness and pain in the spine and limbs The liver was somewhat enlarged The skull was bulged in the right temporal area, and a tantalum plate had previously been fitted after an operation for subtemporal de compression The patient was well nourished, intelligent and co operative She stood and walked with a stoop, and had pain and difficulty in moving (Plate 37) X-ray studies indicated extensive skeletal abnormalities, inclu ding calcification of the ligamenta flava, adjacent to the spine, and of the infrapatellar ligaments (Plate 38) In the course of extensive biochemical studies, however, the level of calcium in the blood was found to be normal

The diagnoses which had previously been made included brain tumour chronic encephalitis, viral radiculoencephalitis, psychoneurosis and gener alised infectious arthritis Her strange clinical picture led to investigations to exclude Addison's disease, dermatomyositis and hepatitis Sobel and his colleagues, however, were aware of the existence of hypervitaminosis A and enquired whether unduly large doses of the vitamin had been taken This was indeed the case For the treatment of ichthyosis (Chap 31) daily doses of 500 000 i u had been consumed regularly for eight years Chemical examination of the blood plasma indicated a level of 6600 i u per ml, which was claimed as a world record

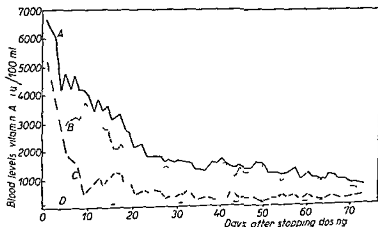


Fig 34 Decrease of vitamin A in the blood plasma of the woman shown in Plate 37 after the removal of vitamin A from the diet Curve A = total vitamin A Curve B = vitamin A esters Curve C = vitamin A alcohol Curve D = normal average level of total vitamin A (Gerber Raab and Sobel 1954)

On discontinuing the excessive dosing with vitamin A the condition of the patient rapidly improved Pruritis ceased after 2½ weeks improvement of the skin was noticed after one month and after two months all spontaneous pain was gone with improvement in the posture

The rate of fall of vitamin A in the blood is shown in Fig 34 It will be seen that the total vitamin A fell rapidly but remained much above the normal average for the whole period of observation Except immediately after the cessation of dosing the excess of vitamin was mainly in the esterified form with the level of alcohol decreasing almost to within the normal range The change of the balance between esters and alcohol soon after the discontinuation of dosing is most interesting and deserves further study

Other cases of hypervitaminosis A in adults have been reported by Sulzberger and Lazar [24] and by Bifulco [25]

Hydrocephalus in infants The occurrence of hydrocephalus in Sobel s case recalls earlier work by Marie and See [26] [27] In 1951 these workers noticed that when infants were dosed with about 300 000 i u each of vitamins A and D_2 they developed about twelve hours later a mushroom like protuberance of the fontanel accompanied by vomitting The hydrocephalus was induced by a single dose of the vitamins and subsided either spontaneously or through lumbar puncture within 24–48 hours after dosing The fluid obtained by puncture was clear and contained no vitamin A Later it was found that the hydrocephalus could be induced by natural vitamin A without vitamin D_2 but the surprising claim was made that synthetic vitamin A does not cause hydrocephalus [28] The hydrocephalus resulted in no permanent injury Its occurrence and association with overdosage with vitamin A was confirmed by many other workers [29] [34]

Through the kindness of Dr Solomon Schwartz of New York [35] the author is able to mention two cases of a more chronic type of hydrocephalus which resulted from prolonged overdosage with vitamin A in young children In a three years old male child who had been admitted to hospital with a history of pruritis and painful legs the skull was found to be large and X ray examination revealed sutural diathesis (Plate 39) Questioning revealed that one teaspoonful of percomorph oil equivalent to 250 000 i u of vitamin A had been taken daily since infancy Biochemical studies indicated a level of over 6000 i u of vitamin A per 100 ml thus equalling the record established by Sobel s adult The child made a good recovery after vitamin A had been removed from the diet and a year later X ray examination showed the skull to be normal (Plate 39) The other case in a two year old male was essentially similar

References p 454

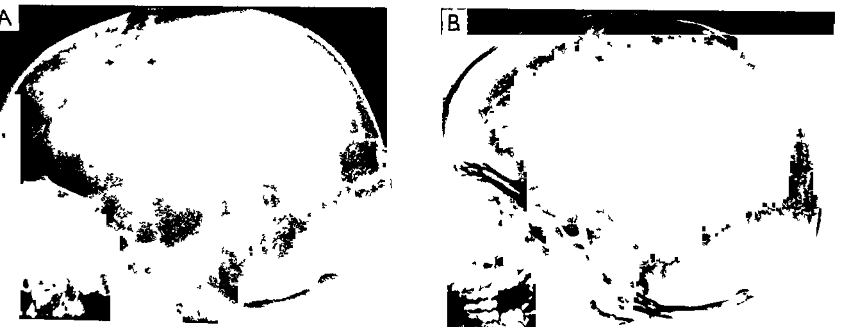


Plate 39 Hydrocephalus in a 3 year old child which had been dosed since infancy with 250 000 i u of vitamin A daily A When first examined Note the sutural diathesis B One year after dosing with vitamin A had been stopped Note the closing of the sutures (Schwartz)

Bear liver poisoning

It has long been known among Eskimos and Arctic travellers that the consumption of polar bear liver by man and dogs can cause severe illness According to Richardson[36] the members of an expedition led to Novaya Zembla by Barentszoon as early as 1596 all became ill when they ate bear liver In three cases the illness was severe with loss of skin from head to foot Another early account of the phenomenon was given in 1856 by Kane[37] who experimented with bear liver with varying results On two occasions the whole company on a journey became sick after eating bear liver but at other times there were no ill effects An account by Koettlitz[38] told how several members of an English expedition to Franz Josef Land in 1894 7 ate polar bear liver and all suffered in consequence

Lindhard[39] reported poisoning among the members of yet another expedition A bear was shot which appeared to be healthy although somewhat thin and on the following day a stew was prepared from the liver heart and kidneys Although the hearts and kidneys of bears had often been eaten before without ill effects the nineteen men who partook of the stew on this occasion all became sick The first signs of distress occurred in two victims 2 4 hours after the meal and most others became ill during the night The symptoms described were drowsiness sluggishness irritability or an irresistable desire to sleep and severe headache and vomiting During the second 24 hours the skin of ten of the patients began to peel around the mouth beginning in spots and gradually spreading over larger areas In some cases the peeling was confined to the face but in others it was general

Lindhard also described three other cases in which the skin peeled from head to foot after bear liver had been eaten On the other hand the Norwegian explorer Nansen[40] mentioned that on two occasions he ate bear liver without ill effects The quantities which he consumed were admittedly small and presumably not enough to injure him The most recent cases of poisoning were described in 1940 by Doutt[41]

The suggestion that bear liver poisoning is a form of hypervitaminosis A was made by Rodahl and Moore[42] after analysis of specimens obtained by Rodahl in Greenland had demonstrated the very high concentration of vitamin A which it contained In specimens from three polar bears values of 13 000–18 000 i u per g were found by the antimony trichloride method and were confirmed by a few biological tests The liver was administered with some difficulty to experimental rats and was found to produce the characteristic lesions of hypervitaminosis A (Chap 28)

It cannot be excluded of course that polar bear liver may still contain unidentified toxic substances It is clear however that its high vitamin A concentration in itself could not fail to make it toxic if eaten in large

References p 454

amounts Thus the consumption of 500 g of liver, by no means an excessive meal for a hungry explorer, would provide about 9 000 000 i u of vitamin A, or nearly 20 times the total reserves which might be considered typical for a healthy human adult It is understandable therefore, that the effects of unwise indulgence in bear liver should include vomiting as seen in infants after a single massive dose of the vitamin rather than bone lesions as seen after prolonged chronic overdosing The occurrence of skin lesions seems to be consistent with the pruritis and skin abnormalities in children and with somewhat similar lesions in experimental rats

According to Rodahl and Moore [42] there have been reports that the liver of the bearded seal *Phoca barbata*, resembles that of the polar bear in being poisonous Examination of a single specimen revealed another rich source of vitamin A with 13 000 i u per g

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PART VIII
SPECIAL TOPICS

CHAPTER 34

Vitamin A in Farm and Domestic Animals

Knowledge of the vitamin requirements of domesticated animals, and of the means by which they may be satisfied, is obviously of great practical and economic importance Animals inadequately supplied with vitamin A, either preformed or as its provitamins, will fail to thrive, and so cause wastage of the food, labour and land which are devoted to their production. There may also be deterioration in the quality of animal products used as human food Thus milk obtained from cows which are partially deficient in the vitamin or eggs from hens in a similar condition, will not supply the consumer with the contribution of vitamin which he might rightfully expect from these foods

The problems that have to be faced in ensuring adequate supplies of vitamin A for animals will obviously depend on the species, and also on the methods of management employed As pointed out in Chapter 13 herbivorous animals receive a great surfeit of carotene while they are subsisting on their normal diet of herbage Inadequate intakes are therefore to be expected only during feeding on substitute rations which are low in the vitamin, or during grazing on pasture which has been parched by draught Under practical conditions of farm management real danger of acute deficiency can arise from either of these circumstances Blaxter[1]

In animals which are non-herbivorous, or which partake only occasionally of small amounts of grass, the problem of supplying vitamin A is much more urgent, and must be faced throughout the life of the animal. Unless the rations of poultry and pigs contain some source of vitamin A activity, such as cod-liver oil or dried grass meal, symptoms of vitamin A deficiency can be developed all too readily. In pet dogs and cats there is little evidence of deficiency, although their sources of vitamin A must often be slender and

References p 494

precarious Those who make a business of breeding these animals of course are usually careful to ensure that an adequate source of the vitamin is given

It will be convenient first to review certain information which concerns all animals and then to deal with each species in somewhat greater detail

Vitamin A Reserves in Different Species

Typical rounded values for the liver reserves of vitamin A in various domesticated animals have already been given in Chapter 13 For additional information in the form of the averages actually found in numerous estimations we may refer to two investigations which were carried out with the main purpose of making comparisons between species

The results of an extensive study by Harms [3] are given in Table 53 For the more familiar animals with the exception of bovines the average vitamin A reserves agree well with the experience of other investigators Thus pigs had lower reserves than the herbivorous animals and guinea pigs had much lower reserves than rabbits Young animals including calves lambs piglets foals chicks and ducklings had all reserves much below the levels found in adults For bovines however, the average was of the same order as for sheep in contrast to the marked superiority found for the sheep by most other workers The reserves found in foxes which were presumably from a fur farm may be compared with values of 600–2050 i u per g mean 940 i u found by Davies and Worden [4] for five wild red foxes

TABLE 53

AVERAGE VITAMIN A RESERVES IN THE LIVERS OF FARM STOCK DOMESTIC ANIMALS AND POULTRY (HARMS 1942)

	No of animals	*Average vit A i u /g*		*No of animals*	*Average vit A i u /g*
Cattle	25	618	Guinea pig	11	71
Calf	44	121	Beaver	11	105
Sheep	19	503	Mink	2	1884
Lamb	14	66	Silver fox	19	213
Goat	5	519	Red fox	3	759
Pig	28	85	Hen	322	905
Piglet	22	18	Chick 1–4 wks	24	61
Horse	20	483	Duck	20	247
Foal	33	18	Duckling	24	7
Dog	3	181	Goose	20	329
Cat	8	145	Turkey	19	73
Rabbit	74	186	Dove	20	408

Moore and Payne [5] examined livers from sheep bovines and pigs which were supplied by a butcher who knew the history of the animals From

Table 54 it will be seen that the low reserves in pigs were again confirmed In contrast to the findings of Harms however lower reserves were found for cattle than for sheep This disparity is perhaps made possible by a greater variation among the reserves of cattle than among those of sheep Thus Moore and Payne found a range of 6 450 i u per g for 36 adult bovines as compared with 190–825 i u for sheep For eleven steers killed during winter an average of only 34 i u per g was found It seems probable that the wide range of variation in bovines may be due in part to dietary differences While bovines are often stall fed sheep usually remain on pasture It may be suspected therefore that the livers examined by Harms were mostly taken from bovines which had been at pasture

Interesting observations by Stewart[6] however suggest that our knowledge of the factors which determine the magnitude of the vitamin A reserves in the bovine liver is still incomplete In a study of the seasonal variation in the reserves of animals killed in a Scottish slaughter house the highest values were observed in winter and the lowest in early summer The cause of this unexpected finding is still obscure

TABLE 54

AVERAGE VITAMIN A RESERVES IN THE LIVERS OF BOVINES SHEEP AND PIGS (MOORE AND PAYNE 1942)

	No of animals	*Vitamin A i u /g*
Sheep	20	460
Bovines adult	36	151
Calves 3 days 6 months old	25	63
Pigs adult sows	6	100
Pigs 6–8 months old	14	22

Vitamin A Requirements

Important studies on the vitamin A requirements of cattle sheep and swine were described in 1937 by Guilbert Miller and Hughes[7] The animals were kept on diets low in vitamin A until they had become night blind as demonstrated by their inability to avoid obstacles when they were driven round their enclosures in semi darkness They were then dosed with vitamin A or carotene in varying doses until the amounts necessary to restore and maintain normal dark adaptation were found These doses were given for periods of a few weeks up to several months Finally the reserves of vitamin which had been accumulated during dosing were assessed either by chemical estimation or by finding the time which elapsed before dark

References p 494

adaptation had again deteriorated The results of the investigation were later reviewed by Hart [8], who made allowances for recalibration of the cod-liver oil which had been used as a source of vitamin A. The requirements were given as follows

	Daily requirement per kg of body weight i u	
	Vitamin A	*Carotene*
Cattle	21–27	43–55
Sheep	17–26	42–58
Swine	18–24	42–65
Horse	17–22	33–50

It will be seen that in all animals the requirements for carotene, expressed in international units, were more than twice those for vitamin A. For either vitamin A or carotene, however, the requirements of the four species covered much the same range. Hart endorsed the conclusion that the vitamin requirements of animals of different sizes are proportional to their body weights, and not to the energy expenditures In Chapter 20, it may be recalled, the author has already given his reasons for favouring the contrary view The distinction can probably be neglected when we are comparing the requirements of farm animals of the same order in size It becomes important, however, when we are comparing the requirements of farm animals with those of small laboratory animals, such as the rat.

VITAMIN A IN THE BOVINE

The literature on the necessity of vitamin A for cattle, and on the vitamin contents of various dairy products, is too extensive to be reviewed in detail in a book not specially devoted to this single field In this chapter space can be found to mention only a small selection from the numerous communications which have been published in all parts of the world Fortunately the task of condensation will be lightened by part of the field having been covered in other chapters Thus Lane Moore's important observations on malformation of the bones in vitamin A deficiency have been described in Chapter 26 The secretion of vitamin A in colostrum and milk has been covered in Chapter 21

Vitamin A deficiency in adult or adolescent bovines

Symptoms of "fat sickness", which presumably were due to deficiency of vitamin A were described as early as 1896 by Connell and Carson [9] The condition was characterised by inflammation of the eyes, or by total loss of sight Hart, Steenbock, Humphrey and Hulce [10]



Plate 40 Symptoms of vitamin A deficiency in a Shorthorn steer after restriction for 309 days to a diet inadequate in vitamin A The steer was blind and rough coated, but had made fairly good gains in weight *Above* Note slobbering Rapid respiration was common and the animal panted after slight exertion in hot weather *Below* Note the drawing back of the head during a typical attack of convulsions The fracture of the left horn seen above was sustained during such an attack The appetite began to fail after the 300th day of the experiment and the animal died in convulsions on the 343rd day

It is an interesting point that the diet contained 80% of yellow maize The inadequacy of this source of provitamins was confirmed by the very low levels of vitamin A and carotenoids which were found in the blood plasma (Madsen and Earle 1947)

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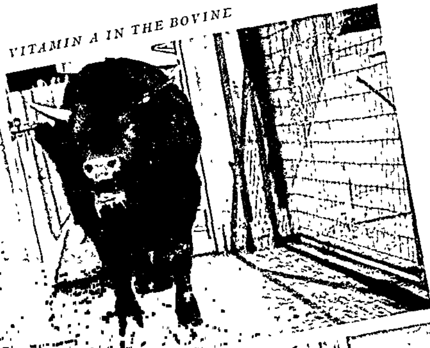
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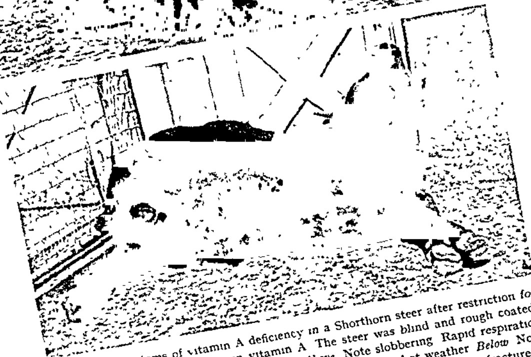


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Plate 40 Symptoms of vitamin A deficiency in a Shorthorn steer after restriction for 309 days to a diet inadequate in vitamin A The steer was blind and rough coated but had made fairly good gains in weight *Above* Note slobbering Rapid respiration was common and the animal panted after slight exertion in hot weather *Below* Note the drawing back of the head during a typical attack of convulsions The fracture of the left horn seen above was sustained during such an attack The appetite began to fail after the 300th day of the experiment and the animal died in convulsions on the 343rd day

It is an interesting point that the diet contained 80% of yellow maize The inadequacy of this source of provitamins was confirmed by the very low levels of vitamin A and carotenoids which were found in the blood plasma (Madsen and Earle 1947)

emphasized the importance of vitamin A for bovine health in 1924, and soon afterwards the experimental production of deficiency in calves and heifers was described by Jones, Eckles and Palmer[11], and by Bechdel, Honeywell and Dutcher[12]. Hart and his colleagues[13, 14] considered that a low vitamin A content in the parched herbage of rocky Californian plains might be one of the factors concerned in the production of stunted, short-legged calves. Since the herbage was also deficient in protein, however, it was obviously desirable to explore the effects of uncomplicated vitamin A deficiency. Guilbert and Hart[15] therefore kept steers on a diet of dried sugar beet pulp, rolled barley, cottonseed meal and calcium carbonate for about 9 months. The signs of avitaminosis A were night blindness, discharge of [illegible] from the eyes, facial paralysis and muscular [illegible] loss of appetite and roughness of the skin were also noticed. In the later stages of deficiency the steers failed to thrive, and lost weight rapidly. Estimations by the antimony trichloride method indicated the presence of only traces of vitamin A in the liver.

In similar experiments by Madsen and Earle[16] anasarca, or generalised oedema, was one of the results of prolonged deficiency. This condition had already attracted attention as a cause for the rejection of carcases by American meat inspectors. In a typical experiment a steer first had convulsions after 6 months of deprivation, diarrhoea after 8 months, and blindness after 9 months. The appetite then declined, oedema ensued, and the animal died in convulsions after 11 months (Plate 40). Other symptoms included protruding eyes and lameness. Photographs of deficient bovines, kindly supplied by Dr. Lane Moore, are shown in Plates 41 and 42.

According to Jungherr, Helmboldt and Eaton[17] the most consistent changes in deficient bull calves were seen in the parotid glands, in the form of squamous metaplasia of the interlobar ducts. These workers again confirmed the early occurrence of convulsions, and of the frequency of impaired eyesight and exophthalmos. It seems probable that the convulsions are an effect of the raised pressure in the cerebrospinal fluid, which may in turn be associated with malformation of the skull bones, as observed by Lane Moore and Sykes[18]. Fig. 35 shows the inverse relationship between the cerebrospinal fluid pressure and the level of carotene in the blood plasma in a heifer which received an inadequate allowance of carotene.

Cystic pituitary glands were observed by Madsen, Hall and Converse[19] in young cattle which were either suffering from vitamin A deficiency or had a history of severe depletion early in life. The cysts occurred either in the residual lumen or in the posterior lobe, and often caused compression of the gland and injury to the glandular parenchyma. No evidence of repair was found in animals which had been deficient in the vitamin early in life





Plate 41 Vitamin A deficiency in a Jersey bull *Above* Although blind the animal was in good physical condition *Below* Head of the same animal showing exophthalmus (U S Dept Agriculture Beltsville)

and had later been dosed with carotene, which suggests that the injury is permanent.

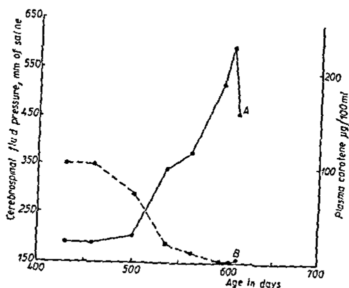


Fig 35 Increasing cerebrospinal fluid pressure contrasted with decreasing plasma carotene in a heifer which had received a diet inadequate in carotene since an age of 120 days A Cerebrospinal fluid pressure B = Plasma carotene (L A Moore and Sykes 1941)

Delayed development of blindness

Experience in England has confirmed the American incrimination of the excessive feeding of sugar beet pulp as a frequent cause of vitamin A deficiency The author was privileged to assist the late Professor F Blakemore in research on this topic, which confirmed that the vitamin A status was low in several bovines which had become blind while receiving the pulp In other animals however, the liver reserves were found to be normal at the time of death In these cases enquiry into their history usually revealed that the animals had been fed upon sugar beet some months previously It seems probable, therefore that the actual breakdown in the optic nerve may sometimes be delayed until growth, under normal nutritional conditions has imposed a further stress on the primary anatomical lesion The development of blindness in calves fed upon sugar beet pulp and the underlying injury to the nerve were confirmed under experimental conditions [20]

Defective reproduction

In 1932 Meigs and Converse [21] reported that when dairy cows were kept on a diet containing inferior roughage and presumably deficient in carotene, they gave birth prematurely to dead weak or blind calves A photograph of such a calf again supplied by Dr Lane Moore, is shown in Plate 43 Soon afterwards Hart and Guilbert [14] reported that in an outbreak of vitamin A deficiency, caused by parched herbage, 75% of the cows under observation failed to carry pregnancy to a successful completion The weak calves produced by the

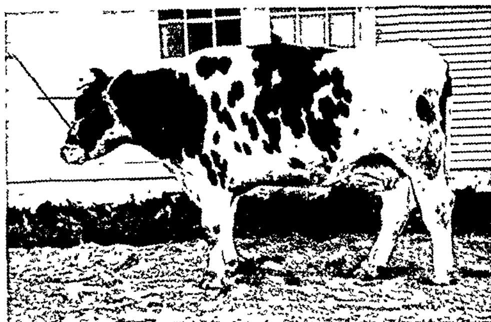




Plate 42 Vitamin A deficiency in a mature cow which was night blind *Above* Note oedema (anasarca) of the hind legs *Below* Close up photograph of the hind legs of the same animal (Michigan Agric Exp Station)

remaining animals developed diarrhoea and all died within 1–5 days after birth In the experience of Davis and Madsen [22] daily intakes of 30–45 μg of carotene per kg of body weight were insufficient to allow cows to produce healthy calves Doses of 60 μg however were adequate



Plate 43 Calf born blind and weak or partially paralysed The dam had received a ration deficient in vitamin A (Michigan Agric Exp Station)

Remarkable abnormalities were reported by Schmidt [23] in some of the calves produced by cows which received inadequate amounts of carotene Thus a profusion of hair was found to be growing on the cornea which gave the impression that the eyeball was continuous with the skin One animal had a deformed foot *These observations suggest that maternal deficiency* of vitamin A can cause congenital abnormalities in bovines as in other animals (Chap 27)

In bulls given a diet low in vitamin A Madsen *et al* [24] found that the production of spermatozoa was continued even after night blindness and muscular incoordination had clearly demonstrated a state of deficiency Sperm from such animals could be used successfully for artificial insemination More prolonged deficiency however was found by Guilbert and Hart [25] to cause degeneration of the seminiferous tubules It appeared that the lesions were reversible since bulls which had been cured of severe deficiency regained normal potency

In carefully planned experiments Bratton *et al* [26] fed six relatively mature bulls of weights 1083–1776 lbs on a diet containing a concentrate and either hay or straw The concentrate contained no carotene the hay 1 27–4 24 mg per lb and the straw 0 56–1 80 mg per lb Over a period of sixteen months there were no signs either of vitamin A deficiency or of impairment

in semen production Sugar beet pulp was then substituted for the hay or straw, and three of the animals were given supplements of carotene, in oily solution, at the rate of 30 mg per 100 lbs of body weight daily During the next five months the bulls which were not given carotene developed incoordination oedema of the extremities and papillary haemorrhage The

production, and the animal retained an unusual degree of libido even after they were unable to stand Histological examination of the testes after slaughter revealed degeneration of the germinal epithelium of the seminiferous tubules with few spermatogonia, spermatocytes, spermatids or maturing spermatozoa in the lumen of the tubules The bulls dosed with carotene remained free from abnormalities Frequent tests on the blood of the dosed and undosed animals confirmed the differences in their levels with the observation of the following ranges

	Undosed bulls	*Dosed bulls*
Vitamin A i u /100 ml	7–10	73–122
Carotene μg/100 ml	8–18	163–400

These findings again emphasise the danger of sugar beet pulp as a cause of vitamin A deficiency under practical farming conditions

The vitamin A status in young calves

In Chapter 21 we have already mentioned that the calf is born with only low concentrations of vitamin A in its blood and liver, and that these scanty supplies are augmented soon after birth if access to the colostrum is allowed. Most practical dairy farmers are aware, moreover, that denial of the colostrum involves grave risks, at least in certain breeds Thus a form of diarrhoea, known as "white scour", usually ensues and often proves fatal within a few days

Observations by the author in a typical experiment on the level of vitamin A in the blood of two newborn calves, only one of which was allowed colostrum, are shown graphically in Fig 36 It will be seen that the vitamin rose steeply in the calf which was given colostrum, and that the animal survived throughout the period of observation In the other calf the original low level of vitamin was not increased, and death occurred at an age of three days It is obvious therefore that the ingestion of the colostrum can both increase the vitamin A status and permit survival

In spite of this correlation, however, it has been clearly demonstrated that the constituent in colostrum which is reponsible for the survival of the calf, during the first few days of its life, is not vitamin A Thus as early as 1922

References p 494

Smith and Little [27] demonstrated that calves receive antibodies from their mother via the colostrum, and claimed that these antibodies gave protection

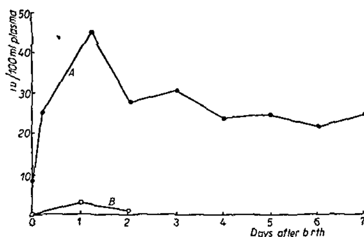


Fig 36 Effect of colostrum on the level of vitamin A in the blood of newborn calves A = Calf allowed access to colostrum B = Calf not allowed colostrum (Blakemore and Moore unpublished)

against diarrhoea In newborn calves but not in calves a few days old the intestines are permeable to the passage of intact protein Soon after Smith s discovery Howe [28] suggested that the antibodies are associated with the globulin fraction of the colostrum More recently Aschaffenburg [29] has devised a simple chemical test, depending on turbidity in the plasma after treatment with zinc sulphate, which tells plainly whether a calf has been allowed or denied access to the colostrum

In spite of Smith's impressive findings the subsequent discovery by Dann [30] of the high concentration of vitamin A in bovine colostrum suggested the alternative possibility that the vitamin and not antibodies, might be mainly responsible for its protective powers In favour of this view Stewart and McCallum [31] found that the liability of calves to white scour, navel ill and joint ill was to some extent dependent on the vitamin A content of the colostrum Thus the incidence of these infections in calves which had received colostrum containing less than 250 "blue units' per 100 ml was 26 8%, as compared with 9 3% when the colostrum had contained more than 250 units per 100 ml Phillips and his colleagues in Wisconsin claimed that the administration of massive doses of vitamin A to calves could prevent white scour and prolong survival [32–34]

In the experience of most other workers, howevers, vitamin A is ineffective in prolonging the life of newborn calves [35 38] With Blakemore and other colleagues [39] *the author confirmed* that calves usually developed fatal ' white scour" when they were denied colostrum and were kept on a diet of *whole or* separated milk Concentrated preparations of vitamin A, administered in

various forms gave no protection Survival over the early critical period was achieved, however, by giving injections of pre colostrum a glutinous liquid which appears in the udder long before parturition This material is rich in globulins but contains relatively little vitamin A Extensive experiments by Kon and his colleagues [40-49] gave further evidence of the immunological importance of colostrum Thus the fatty fraction separated from colostrum by centrifugation did not prevent early death, whereas the aqueous phase was always effective Even single doses as low as 80 ml allowed survival although the animals scoured severely and gained little weight

While accepting the importance of globulins, however, we must remember that adult bovines are liable to suffer from the effects of avitaminosis A when they are kept on a deficient diet for long periods It seems reasonable to conclude therefore, that young calves, possessing only scanty reserves of the vitamin at birth will be even more vulnerable to deprivation of the vitamin The findings of Stewart and McCallum [31] on the inferior protective power of colostra low in vitamin A, which we have no reason to dismiss, at least suggest that vitamin A is necessary to supplement the action of the globulins

It is a difficult problem however, to obtain proof of suspicions that a young animal is inadequately supplied with vitamin A The observation of a very low level of the vitamin in the blood or liver may indicate no more than the point which would normally have been reached in the slow increase from neo natal up to adult levels A failure to thrive, at a stage too early for clear indications of avitaminosis A to be expected, may merely be an after effect of an earlier infection The experimental productions of vitamin A deficiency in young calves provided with the antibodies but not the vitamin A of colostrum should repay further careful study

We have seen in Chapter 18 that the efficiency of absorption of vitamin A may be greatly influenced by the form in which it is administered The effect of emulsification may be demonstrated very readily in the calf Thus in experiments by the author s colleague Dr S E Walker [50], three calves were dosed on separate occasions, with 10 ml of halibut-liver oil mixed with 15 ml of arachis oil On one occasion the mixture, which provided about 500 000 i u of vitamin A, was given orally to the calves without further treatment On the other occasion the oils were emulsified with the aid of 250 ml of milk and about 80 g of wheat flour From the average levels of vitamin A found in the plasma at intervals after dosing, as shown in Fig 37, it will be seen that the emulsification greatly accelerated the absorption of the vitamin

Blakemore and his colleagues [39] reported that newborn calves can absorb

References p 494

vitamin A although the assimilation of the vitamin does not prevent the subsequent onset of diarrhoea Once the scouring has started the absorption of the vitamin becomes defective

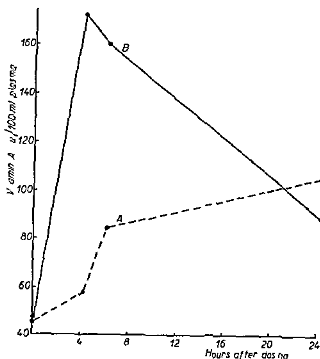


Fig 37 Effect of emulsification on the absorption of vitamin A by calves (A) Single dose of 500 000 i u of vitamin A given as plain halibut liver oil (B) 500 000 i u of vitamin A given as halibut liver oil but emulsified with a little wheat flour and milk (Walker 1947)

Requirements for milk production

The vitamin A requirements which were established for bovines by Guilbert and his colleagues [7] appear to relate mainly to animals used for beef production An additional allowance seems necessary for lactating cows which have both to maintain their own health and secrete vitamin A and carotene into their milk An exact assessment of the additional requirement is difficult and its magnitude is greatly influenced by our intention in making the additional allowance If our desire is merely to replace vitamin A which is lost in the milk the demand may be fairly small On the other hand if it is intended to raise the level of vitamin A in the milk to satisfy some agreed standard very massive dosing may be necessary

Atkeson *et al* [51] have assessed the combined output of vitamin A and carotene in the milk of a pasture fed cow as about 13 000 i u daily If we assume that the animal weighs 500 kg its requirements for maintenance according to Guilbert will be about 500 × 50 i u of carotene or 25 000 i u daily As a book keeping transaction therefore it seems only necessary to increase the maintenance allowance by some 50% in order to compensate for the losses incurred by milk production

Complications arise, however, when we desire to raise the vitamin contents of the milk to a fixed target As already mentioned (Chap 13) the utilisation of carotene by the cow, at least when it receives large quantities, is extremely inefficient. Atkeson *et al* calculate that their cows were ingesting no less than 3 5 g of the provitamin daily, and that the combined secretion of vitamin A and carotene in the milk accounted for only 0 24% of the intake The proportion of an extra dose of carotene which is actually used for the intended purpose of enriching the milk, therefore may sometimes be as low as one part in 500

If he attempts to theorise, therefore the dairy farmer finds himself between the horns of a dilemma If he merely gives his cows an allowance numerically equal to the amount secreted he has no assurance that inefficient absorption will not make his attempt at compensation inadequate If he attempts to reach fixed high targets for the levels of vitamin A and carotene in the milk he may feel that the small increases in the milk hardly justify the much greater increases in the dietary intake which were required to produce them In practice he will generally compromise by allowing his cows access to pasture during the summer, and by providing them with reasonable allowances of grass or alfalfa meal silage or good quality hay in the winter The concentration of vitamin in the milk can then be left to find its own level

X disease (hyperkeratosis) in cattle The discovery in America of this new disease in cattle has been important for reasons concerning both practical dairy husbandry and our theoretical knowledge of the mode of action of vitamin A The condition was observed by Olafson [52] in 1941 among cattle in New York State, but was not reported until 1947 He describes the first two heifers which were noticed to be suffering from the complaint as emaciated thick skinned and hide bound (Plate 44) In spite of their emaciation they refused food The condition had the appearance of mange but no mites could be detected Numerous further cases followed mainly in animals 3–12 months old and during the late winter and spring Post mortem examination indicated that the animals had also internal abnormalities Papillary proliferation was found in the mouth and stomach The bile ducts were dilated, with proliferation of their linings The gall bladder was lined with cyst-like blebs, which contained mucus Fibrosis was found in the liver, and cystic dilation of the straight tubules in the kidneys The disease could not be transmitted to other bovines

In Olafson's experience badly affected calves could not be cured by dosing with either vitamin A or with B vitamins The proliferation and keratinisation of epithelial tissues however, was obviously suggestive of avitaminosis A Estimations of vitamin A were in fact made by Dr J K Loosli,

References p 494

but the results which appear to have been low were at first presented without comment A similarity of the disease to Wendener Krankheit observed in Germany by Gminder [53] was pointed out



Plate 44 X disease (hyperkeratosis) as caused by chlorinated naphthalenes in a calf Note the marked thickening and wrinkling of the skin with loss of hair on the upper two thirds of the body The case was observed during one of the first outbreaks of the disease (Olafson 1947)

The first hint as to the cause of the illness came when Olafson and McEntee [54] succeeded in inducing the condition in experimental calves and also in sheep and guinea pigs by feeding them on a processed wheat concentrate from the same source as had been eaten by animals affected in the field In calves weighing 200–400 lbs the administration of about one litre of the concentrate daily for about two weeks caused the production of all the expected abnormalities Moreover the disease continued to progress even after the feeding of the concentrate had been stopped and caused the death of the calves after an interval of several weeks Soon afterwards it was found by Hansel McEntee and Olafson [55] that a toxic fraction could be extracted from the concentrate with ether leaving a harmless residue

The same workers [56] made extensive studies on the effect of the hyperkeratosis on the vitamin A status The condition was induced either by processed wheat concentrate as before or by the topical or oral administration of a wood preservative which had been found to produce hyperkeratosis by Wagener [57] in Germany When the calves were housed in stalls

which had been treated with the preservative they developed hyperkeratosis on the part of their body which had been rubbed against the stall Their skin lesions were worse than in animals which had been given the preservative orally, but their general condition was better The level of vitamin A in the blood plasma was depressed both by the preservative and by the wheat concentrate (Fig. 38) The decrease in vitamin A caused by oral treatment with either toxic agent, however, was greater than the decrease caused by the external application of preservative The declines in vitamin A persisted for long periods after treatment with the toxic substances had been stopped Massive dosing with vitamin A temporarily raised the levels in the blood, but low values were again observed after dosing had been discontinued

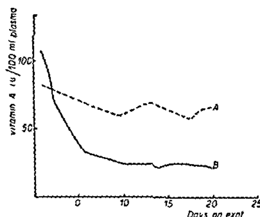


Fig 38 X disease The effect of experimentally produced hyperkeratosis on the level of vitamin A in the blood plasma of calves (A) Average curve for three untreated healthy calves (B) Calf suffering from hyperkeratosis induced by feeding upon a processed wheat concentrate (Hansel McEntee and Olafson 1951)

A vital clue was next provided by Sikes and Bridges [58] who found that hyperkeratosis could be induced experimentally in cattle by oral treatment with a chlorinated naphthalene, pentachloronaphthalene Bell [59] produced the disease by the administration of a lubricant, in which the presence of chlorinated naphthalenes might have been expected Olafson, McEntee and Hansel [60, 61] isolated both a chlorinated naphthalene and trichlorobenzene from the German wood preservative, and found that both were toxic. Engel and Bell [62] commented on the presence of chlorinated naphthalenes not only *in motor oils but also in materials used for the insulation of electric cables* A form of acne in humans was known to result from their use

It now became clear that hyperkeratosis could be induced in various ways, but that the most frequent cause was the inclusion of chlorinated naphthalenes in mineral oils used for the manufacture of animal foodstuffs. In a crucial experiment Copenhaver and Bell [63] had batches of pellet feed

References p 494

made either with plain lubricant oil, or with oil to which 3% of chlorinated naphthalenes had been added In feeding trials with calves hyperkeratosis was only developed in those animals which received the pellets which had been contaminated with the chlorinated naphthalenes

Another source of danger, however, was pointed out by Hoekstra, Dicke and Phillips [64] who demonstrated that hyperkeratosis could arise from the use of harmful insecticides Skin lesions and decreased vitamin A levels could be induced by spraying calves with oils containing either chlorinated substances or thiocyanate derivatives This last finding, which obviously deserves further investigation, raises an important point Can X-disease be regarded as a specific effect of one particular type of poison? Or could a wide range of substances produce the same effect, if they were allowed to contaminate the food or living quarters of cattle?

Ferrando [65] has suggested that X disease may be caused not by a direct antagonism between vitamin A and chlorinated naphthalenes, but by the strain placed by the naphthalenes on detoxicating mechanisms This in turn may cause an increased demand for vitamin A, as shown by work by Meunier, Ferrando and Perrot-Thomas [66] on the detoxication of bromobenzene Discussion of implications of this suggestion, which apply to other animals besides bovines, must be postponed until Chapter 38 In the meantime the practical importance of steps to avoid X-disease must be emphasised Thus according to Sikes and Bridges [58] no less than 2300 cases of the disease were recorded in Tennessee for the year 1947 The most important prophylactic measure seems to be the prevention of access of the animals to the toxic substances In the experience of two groups of workers [64, 67] massive dosing with vitamin A is beneficial, but ineffective in either completely curing or preventing the disease

SHEEP

As far as the author is aware there have been no authentic instances of vitamin A deficiency occurring spontaneously in sheep This apparent immunity of the species may probably be associated with the efficient accumulation of reserves of the vitamin, a point to which reference was made earlier There is ample evidence, however, that sheep may be made deficient in the vitamin by experimental methods

Experimental deficiency In 1937 Guilbert, Miller and Hughes [7] described an investigation in which twenty one 7-year-old ewes were given a diet which consisted mainly of sugar beet pulp, with smaller amounts of rolled barley, cottonseed meal and wheat bran The initial reserves appear to have been 600–1000 I U per g, which fell to

50–150 i u after deprivation for 10–16 months The animals which were retained for longer periods developed night blindness after 22–23 months After 27–30 months they died or were killed in a state of cachexia Their vitamin A reserves were now completely depleted Pneumonia enteritis and kidney lesions were the most conspicuous findings at autopsy

Further trials were made by the same workers on a larger group of ewe lambs which developed night blindness after 8–9 months Doses of carotene or vitamin A were then given until the abnormality had been cured with results that have been summarised on page 462

In Australia careful studies by Peirce [68] substantially confirmed the American finding except that the requirement for carotene appeared to be slightly higher Eighteen sheep 4–8 months old were kept on a basic diet of chaffed wheat straw barley linseed meal and a compressed mixture The whole diet supplied only 17 i u of carotene per kg of body weight In animals which were not dosed with carotene the levels of vitamin A in the blood and liver declined as the experiment progressed as indicated by the following averages

Depletion period	*Liver (i u /g)*	*Depletion period*	*Blood plasma (i u /100 ml)*
0	670	0	83
2 mths	340	6 mths	76
4 mths	120	9 mths	43
9 mths	13	13 mths or more	20

Although suffering from night blindness the sheep without supplements grew well for over a year after which most of them lost appetite had muscular incoordination and died The administration of dried lucerne leaves in amounts providing 40–50 i u per kg of body weight to sheep which had been on experiment for 9 months was effective in arresting the fall in the level of vitamin A in the blood plasma This level of dosing however had little effect on night blindness or on the liver reserves and did not prevent muscular incoordination and death in two out of three animals Doses of 82–90 i u per kg given to other animals were effective in raising the vitamin A level in the blood to about 75% of its normal value in curing night blindness and in preventing incoordination and death Only small liver reserves were found in these animals however, when the experiment was terminated after a total period of about 2 years

Later Eveloth Bohn and Goldsby [69] reported the results of yet another large scale investigation Yearling ewes were given a diet of straw, soya bean meal sugar beet pulp and oats After 5–6 months the level of vitamin A in their blood plasma which originally averaged about 100 i u per 100 ml

References p 494

had fallen to about 30 i u The *animals became night blind* and also unusually excitable which made them easily startled and difficult to handle Pica was *evidenced by their desire to chew* fences Some of the sheep died suddenly without showing *symptoms* Others survived longer and developed inco *ordination and lordosis* Evidence was obtained of increased cerebro spinal fluid pressures The vitamin A reserve in an animal which was killed while moribund after restriction for 11 months was only *18 i u* per *g* as com pared with 240–500 i u for lambs on pasture

In a sheep which had been deprived of vitamin A for over two years Schmidt [23] found that the kidneys were encrusted with minerals

Reproduction Frank avitaminosis A induced in ewes by 8 months of experimental deprivation was found by Guilbert Miller and Hughes [7] to have disastrous effects on reproduction Out of eight lambs produced by such mothers seven were weak and died within five days Other workers have shown however that after a summer at pasture the sheep is able successfully to produce its young during winter even if the diet provided is inadequate in the vitamin Thus Weir *et al* [70] and also Colby Cunha and Warwick [71] found that allowances of carotene or vitamin A during winter had no noticeable effect on the lambing performances In Merino ewes which were 4 7 years old and which presumably had high initial reserves of vitamin A Peirce [7] found that lambing only became defective after 19 months of deficiency and after two previous successful matings An allowance of 83 i u of carotene daily per kg of body weight was found to be necessary for the uninterrupted repetition of reproduction

Seminal degradation was found by Gunn Sanders and Granger [73] in night blind rams Lindley *et al* [74] studied the quality of the semen in young rams which had been deprived of vitamin A until they had developed the usual signs of deficiency The testes were undersized and the initial motility of the spermatozoa was reduced An find ng as the frequence of pituitary cysts as ob developed urinary calculi but these were also found in control animals which had been given the vitamin The defects in the testes and spermatozoa could not be corrected either by testosterone or by pregnant mare serum

Pigs

As early as 1914 Hart and McCollum [75] observed stiffness of the hind quarters followed by paralysis in pigs which received wheat kernel as their only food Some of the animals became blind and their general con dition was poor At the time these symptoms were attributed to a toxic factor present in the wheat kernel The possibility of vitamin A deficiency was

suggested, however, by the absence of symptoms when the same diet was supplemented by milk, egg yolk or alfalfa Hughes, Lienhardt and Aubel [76] later demonstrated that the paralysis was in fact due to vitamin A deficiency, which caused degeneration of the nerves

Much early work on vitamin A deficiency in pigs was carried out by Drummond, Golding Zilva and other British workers [77–79], but their studies were complicated by symptoms due to lack of vitamin D, which at that time had not been differentiated from vitamin A

Deficiency in pigs receiving farm rations

In 1934 the author was privileged to assist the late Dr George Dunlop [80] in investigations which demonstrated how readily vitamin A deficiency can occur in pigs when they are given inadequate commercial rations Thus pigs were shown to develop severe vitamin A deficiency, with paralysis, when they were kept upon a mixture of barley meal, middlings and fish meal A diet made up of cereals, soya bean meal and minerals was also defective Such time honoured mixtures were recommended by official agricultural advisers, and their inadequacy came as a shock to practical pig keepers. In retrospect we may guess that pigs at this period must have obtained precarious supplies of vitamin A from grass or garbage, or from dosing with cod liver oil Dunlop tried numerous mixtures, with barley meal, wheatings, rice meal or maize meal as the main constituent Avitaminosis was always liable to develop unless the diet contained an adequate amount of either alfalfa meal, or of yellow maize Estimations of vitamin A in the liver indicated about 15 i u per g when the diet had contained 60% of maize, traces when it had contained 24% of maize or 6% of alfalfa, and no vitamin A at all with other diets, even when 2% of alfalfa was included

Requirements

Guilbert, Miller and Hughes [7] gave pigs experimental diets with components chosen from rolled barley, brewer's rice, wheat middlings tankage, linseed meal, skimmed milk powder and minerals Deficiency was regularly induced and could be cured, without complete correction of the paralysis, by dosing with vitamin A or carotene The findings of these workers for the requirements of the pig have already been given

The precautions taken by Hentges *et al* [81] in finding the carotene requirements of pigs included housing the animals in a dry room kept at 70° F. A deficient diet was first given until the level of vitamin A in the blood serum fell below 23 i u per 100 ml after which the animals were left undosed, or were given graded supplements of pure carotene When the pigs were killed, after a further 7 weeks, the results given in Table 55 were obtained It will be seen that a daily supplement of 17 i u per kg of body weight improved the growth rate, and increased the level of vitamin A in the blood, but

References p 494

allowed no measurable accumulation of vitamin in the liver Slightly higher values for the blood vitamin A and growth were found after doses of 30 i u, but doses of 42 i u were necessary for the appearance of small amounts of vitamin in the liver This dosage agrees well with the lower limit for the requirement found by Guilbert and his colleagues [7] fifteen years earlier

TABLE 55

FINDINGS BY HENTGES *et al* (1952) ON DEPLETED PIGS WHICH HAD BEEN EITHER LEFT UNDOSED, OR HAD BEEN GIVEN GRADED LEVELS OF CAROTENE FOR 7 WEEKS

Daily dose of carotene i u /kg body weight	*Average daily weight gain kg*	*Vitamin A i u /100 ml blood serum*	*Vitamin A i u /g liver*
0	0 22	17	0
17	0 47	44	Trace
30	0 54	60	Trace
42	0 56	79	1 3

In New Zealand Ballinger [82] found that supplements of vitamin A made no difference to the rate of growth, or health of pigs which were fattened on a diet of buttermilk

Reproduction The classical observations of Hale [83] have shown that vitamin A deficiency in pigs can cause gross congenital malformation (Chap 27) When mature sows have been allowed to accumulate reserves of the vitamin, however, subsequent restriction to a deficient diet will not prevent the successful birth of litters Foot, Henry, Kon and Mackintosh [84] gave pregnant sows a diet of barley meal wheatings, extracted soya meal meat meal, and mineral starting two weeks before farrowing was due The young were reared on the same diet, and at first grew fairly normally In the latter stages of fattening, however, the typical symptoms of avitaminosis A were developed Thus appetite was lost growth ceased and vision was impaired in daylight The gait became abnormal, and the hind legs partially paralysed (Plate 45) Convulsive fits and nervous collapse were common Pneumonia, and often inflammation of the intestines were found at autopsy in all the pigs which died For the prevention of these symptoms the inclusion in the diet of 0 5% of cod liver oil was found to be adequate The symptoms also responded readily to curative treatment with vitamin A

In experiments on one particular sow, which had already had five litters Braude Kon, Mitchell and Thompson [85] gained full experience of the experimental difficulties which can arise in attempting to induce deficiency in the offspring of animals having high reserves of the vitamin After

restriction to a deficient diet, consisting of barley meal, wheatfeed and white fish meal, this sow produced three more litters, which were supplied with colostrum rich in the vitamin and successfully reared For a further litter however the colostrum was inadequate in vitamin A, and rearing was unsuccessful Thus the failure of the hepatic vitamin A reserve, confirmed by a low value of 7 i u per g found at autopsy, occurred only after the birth of four litters, during a total period of two years of deprivation

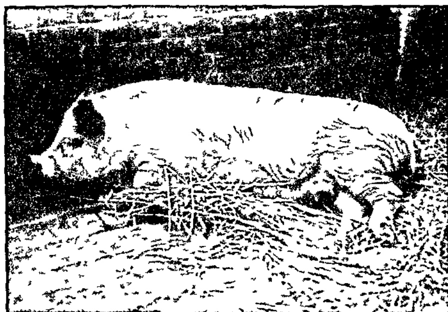


Plate 45 Vitamin A deficiency in a pig Note the position of the hind legs (by courtesy of Dr S K Kon)

In the experience of Parrish *et al* [86] sows and piglets thrived equally well on a deficient diet when the sows were given daily doses of either 6500 i u of vitamin A or 7100 i u of carotene They found however, that vitamin A was more effective than carotene unit for unit in procuring high levels of vitamin in the blood serum and colostrum of the sows, and in the serum and livers of the piglets

OTHER MAMMALS

Horses In the country horses will usually receive adequate amounts of carotene from pasture When they are working in towns, however, they may have to subsist for long periods on diets containing only traces of provitamins Risks of deficiency are also incurred by army horses, particularly in the tropics and by ponies used in mines

References p 494

In 1919 Meadows[87] reported the frequent occurrence of night blindness in horses and camels in Persia He did not know the cause Fourteen years later, Klemola[88] noticed that 10–15% of Finnish cavalry horses, and 20–30% of artillery horses, had brittle hoofs, with furrows and cracks The periople, or front face of the hoof, was scaly, sometimes with the formation of a thick, spongy crust From the nature of the diet, which contained oats, dry hay and oat straw, Klemola concluded that the abnormalities were due to deficiency of vitamin A In support of this view the hoofs regained their normal smooth and shiny appearance when the animals were allowed to graze, or were given silage or cod liver oil Treatment with vitamin D was ineffective

A period followed during which veterinarians were attracted to the possibility that deficiency of vitamin A, and of other nutrients, might be responsible for many common equine abnormalities Hare[89] had already hinted that several common ailments, including ankylosis of the hock joint, might be due to a common systemic lesion Mitchell[90] published a succession of papers, over a period of seven years, in which he elaborated the hypothesis that rheumatic diseases in horses might be due to malnutrition

Chatelain[91] concluded that general debility among French army horses in Indo China was due to vitamin A deficiency, and noticed improvement after their treatment with cabbage pulp, cod liver oil and egg yolk Edwards[92] suggested that inadequate intakes of carotene might be responsible for various forms of unsoundness in horses A high incidence of degenerative arthritis in horses and mules in the Panama Canal zone was attributed by Kesler and Callender[93] to a defective diet of grain, hay and oats

It must be admitted that the evidence supporting some of these suppositions was far from conclusive In 1940, however, Guilbert, Howell and Hart[94] reported an extensive investigation in which the effects of experimental vitamin A deficiency in the horse were clearly demonstrated The animals used were Percherons, mostly 9–12 months old Various diets were given, with their components selected from rolled barley, rolled oats wheat bran, linseed oil meal, cottonseed meal, sugar beet pulp and straw The observation of night blindness was rather difficult, because of the uncanny memory of horses for obstacles, but by special methods its occurrence was established after 8–21 months of dietary restriction The requirements of the species for vitamin A and carotene were then studied by observing the response of the night blindness to dosing, with results which have been given on page 462

Howell, Hart and Ittner[95] later described pathological studies which were based on the same material They listed the abnormalities involved as

night blindness lachrymation keratinisation of the cornea respiratory symptoms capricious appetite progressive weakness and death Lameness was common with rarefying lesions in the joint cartilages Cases of typical string halt and scaly hoofs were observed Breeding was sometimes initiated even after dark adaptation had become defective but the foals seldom survived and one mare died at parturition Howell and his colleagues considered that the evidence that the important joint and bone lesions were a specific effect of avitaminosis A was suggestive but not conclusive They gave a warning that the rations of horses are liable to be seriously deficient in vitamin A unless adequate amounts of yellow maize are included

Anderson and Hart [96] examined the retina taken from the eye of a night blind horse and found vacuolation which appeared to indicate an abnormal accumulation of lipoids This was attributed to an effort on the part of the rods to form rhodopsin In the author s laboratory Rudra [97] estimated vitamin A in the blood serum and liver of about thirty normal horses The values for the serum varied from 6 to 52 i u per 100 ml with a mean of 28 i u and for the liver from 32 to 2000 i u per g with a mean of 628 i u Carotenoids averaged 43 μg per 100 ml of serum Reports that bilirubin also contributes to the yellow colour of the serum in this species [98, 99] were confirmed with an average of 3 1 mg per 100 ml The high liver reserves of vitamin A which are commonly found in horses probably explain their ability to subsist for long periods on deficient diets before the development of night blindness

Goats According to the limited data available goats resemble sheep in having colourless body fat and high reserves of vitamin A in the liver Thus Sen [100] found an average reserve of 565 i u per g for five healthy goats which may be compared with a mean of 519 i u found by Harms

ably prevent stone formation although the incidence of this abnormality was reduced

Dogs The need of the dog for vitamin A has been clearly demonstrated in experimental studies of the pathological effect of avitaminosis A particularly regarding bone formation (Chap 26) As far as the author is aware however there has been no clear evidence that vitamin A deficiency occurs spontaneously in this species Although night blindness is reputed to afflict one particular breed the red Irish setter there is no evidence that a dietary deficiency of the vitamin is involved

Linton and Brownlee [101] estimated vitamin A in the livers of over 100 healthy dogs which had been destroyed at a dogs home Wide variations

References p 494

were found with an average value of 407 i u per g and a median of 93 i u The reserves for dogs which appeared to be only a few months old ranged from 5 to 42 i u per g in contrast to a higher range of 16 to 675 i u in senile dogs No correlation could be found between the magnitude of the vitamin A reserve and either the extent of the fat deposits or the general condition of the animals In seven Belgian dogs Rouir [102] found reserves ranging from 60 to 253 i u per g

The remarkable discovery by Catel [103] that vitamin A is normally excreted in the urine of healthy dogs has already been mentioned in Chapter 32 In seven specimens of urine obtained from four dogs levels of 66–660 i u per 100 ml were observed In confirmation Lawrie Moore and Rajagopal [104] found that numerous random samples of urine from a healthy male Fox Terrier contained from 90 to 450 i u of vitamin A per 100 ml In a normal dog therefore the urinary concentrations of vitamin approached those found in humans suffering from pneumonia Much lower concentrations however have been found in recent work by Worden *et al* [105] In three dogs and one bitch aged 2 6 years and of different breeds vitamin A was always found in repeated examinations of the urine The quantities found ranged from 2 5 to 47 5 i u per 100 ml or 14 3 to 235 0 i u per day It was suggested that the excretion in the urine might be associated with a past history of nephritis which is said to occur commonly in dogs without being recognised Alternatively they considered that the urinary excretion might be in some way connected with high levels of vitamin A in the plasma

Cats Very little seems to be known about the vitamin A status or requirements of cats According to Gillam [106] the liver of a cat contained 108 i u per g while Jensen and With [107] found 380 i u and 520 i u for two animals From Table 53 it will be seen that Harms found an average of 145 i u

Morton and his colleagues Vernon and Cunningham [108] have recently estimated vitamin A in the livers and kidneys of cats For three animals liver reserves of 382 625 and 2320 i u per g were found In nine other cats surprisingly large amounts of vitamin were found in the kidneys with concentrations of 69 to 1341 i u per g mean 98 i u These levels are higher than those commonly found in the liver in some other species such as the pig

Birds

The first proof that birds require vitamin A in contradiction to an early statement that they could dispense with this vitamin [109] was given by Emmett and Peacock [110] and was confirmed by Hart Steenbock Lepkovsky and Halpin [111] It certainly seems strange that the matter was ever

in doubt, since any amateur poultry keeper must expect catastrophies if he neglects to supply his birds with carotene or vitamin A

Effects of deficiency

The most common effects of avitaminosis A in birds are loss of appetite, cessation of growth, incoordi- [illegible] h The eyes are often half [illegible] common feature Capper, [illegible] resembling xerophthalmia in only one chicken in a group of deficient birds, although the "third eyelids" were always partially closed On dissection white deposits of urates are usually evident in the viscera As early as 1921 Beach [113] noticed white streaks on the hearts, livers and spleens of chickens with "nutrition roup", which was attributed to deficiency of vitamin A Emmett and Peacock [110] and Hart *et al* [111] noticed similar deposits and Capper and his colleagues described the condition as "visceral gout" Elvehjem and Neu [114] found pathological changes in the kidneys of deficient chicks which interfered with the excretion of uric acid Seifried [115] focussed attention on the squamous metaplasia which could be observed in the nasal passages of deficient chickens, and Jungherr [116] emphasised the significance of this abnormality, in the absence of other lesions, as an indication of partial deficiency

McClymont and Hart [117] found that egg production ceased in pullets after their restriction for four months to a diet deficient in vitamin A, and about six weeks before they succumbed to the effects of avitaminosis Those eggs which had been laid, however, could be hatched, and no deformities were found in the chicks Meyer [118], on the other hand found that eggs did not usually hatch normally unless they contained at least 350 i u of vitamin A

The occurrence of bone and nerve lesions in ducks deprived of vitamin A has already been mentioned in Chapter 26

Requirements of chickens for vitamin A and provitamins

Early observations by Capper, McKibbin and Prentice [112] suggested that the requirement of the hen for carotene, per unit of body weight, was greater than that of the rat Record, Bethke and Wilder [119] claimed that the growth responses of chicks to carotene and vitamin A were about the same when equivalent amounts were fed at low levels of intake Johnson, Carrick and Hauge [120] found that a high storage of vitamin A in the liver was not essential for satisfactory growth in young chicks

In 1944 a Committee on Animal Nutrition of the American National Research Council [121] gave the requirements of starting chicks as 4400 i u per kg of food, growing chicks 4400, laying hens 7300 and breeding hens 7300 No distinction was made between vitamin A and its provitamins

An extensive investigation by Castano, Boucher and Callenbach [122], which

References p 494

was reported seven years later gave evidence that slightly smaller allowances might be adequate Thus diets containing between 1100 and 2200 i u per kg of either carotene or vitamin A allowed the normal growth of chicks for the first eight weeks of their lives We may note that this finding is lower than the official 1944 estimate but that it does not include a safety margin Allowances of 4400 to 26 500 i u per kg produced no improvement in growth although much larger reserves of vitamin were accumulated in the liver With the highest levels tested 39 700 and 53 000 i u per kg growth was slightly retarded

In studies which covered not only growth but also the laying and hatchability of eggs Leroy and Levy [123] found that allowances somewhat higher than those reported by Castano and his colleagues were necessary There was also a hint that preformed vitamin A unit for unit was more effective than carotene Thus in a first experiment heavy mortality was not prevented by 5000 i u per kg of either vitamin A or carotene The egglaying record however was better with vitamin A than with carotene In a second experiment vitamin A was raised to 10 000 i u and carotene to 20 000 i u Mortalities were now avoided but only 24% of the eggs could be hatched in the vitamin A group and 8% in the carotene group Since considerable improvements could be effected by increasing the vitamin E intake the adequacy of the basal diet must unfortunately come under suspicion

In his book on the scientific principles of poultry keeping Halnan [124] recommends rations in which vitamin A is supplied as 5% of alfalfa meal As an alternative cod liver oil may be included as 2% of chick mash and as 1% of the mash for growers or layers

Requirements of turkeys In the experience of most poultry breeders turkey poults are delicate and hard to rear Possibly this lack of robustness accounts for their unusually high requirement for vitamin A as found by most investigators in this field

The evidence of early studies was somewhat conflicting Scott and Hughes [125] gave growing turkeys vitamin A in the form of yellow maize in amounts which must have supplied about 4000 i u per kg of food and found that this allowance was adequate A much larger intake however was found necessary by Hinshaw and Lloyd [126] who included 17 000 i u per kg of their diet in the form of alfalfa meal Wilgus [127] reported that turkey poults required 6000 i u of crystalline carotene per kg of food which he considered to be four times greater than the requirements for chickens The N R C Committee [121] estimated the requirements of both starting poults and growing turkeys at 8800 i u per l g of food again without distinction between various forms of the vitamin

More recent investigations have shown further inconsistencies in the

requirements found by different workers Thus extensive experiments by Wharton, Matterson Scott and Bliss[128] indicated that young turkeys require 17,000 i u of vitamin A, as cod liver oil, per kg of food to allow maximum growth To prevent a decline in the liver reserves and so maintain a positive balance in the metabolism of the vitamin, an allowance of 12 000 i u per kg appeared to be necessary According to Gurcay, Boucher and Callenbach[129] lower doses sufficed for normal growth Thus poults grew well when they were given about 4400 i u per kg of food if the vitamin were supplied as '*black cod*' liver oil These workers found moreover, that the requirements of turkeys were greatly influenced by the form in which the vitamin was given Thus with crystalline vitamin A acetate the requirement was reduced to 2200 i u but with crystalline carotene it was raised to 8400 i u Much greater liver reserves were accumulated when cod liver was given than when the same numbers of units were supplied as carotene At very high levels of supplementation a twenty fold disparity was found

Van Reen Taylor and Russell[130] agreed that the requirements of turkey poults for growth are relatively low, but commented that higher intakes of vitamin were necessary for normal health than for maximum growth Thus a diet containing 3000 i u per kg of natural vitamin A esters was adequate for normal growth whereas 5000 i u had to be provided to prevent unsteadiness of gait ruffled feathers and an abnormal condition of the inner eyelid Reports by Taylor *et al*[131] were recalled in which histological abnormalities were found in the nasal passages of poults which were receiving the vitamin in even more than adequate amounts at least as judged by the absence of all other signs of deficiency

Russell and his colleagues suggested that the divergence between their findings and those of Wharton *et al* might be due to differences in the breeds of turkeys under investigation which were the light Jersey Buff and the heavy Broad Breasted Bronze respectively As other possibilities we may perhaps suspect that there were differences in the details of management, and in the stability of the sources of vitamin after they had been added to the food On the whole it seems reasonable to agree with Gurcay, Boucher and Callenbach[129] that the vitamin A requirements of the turkey, per unit of diet, are greater than those of the chicken, which are in turn greater than those of the rat In view of incongruities between the results of different workers however, it would seem unwise to attempt to calculate precise ratios for the relative requirements of the three species A summary of findings of various workers for both turkeys and chickens is attempted in Table 56

Requirements for laying During laying the hen must lose about 350 i u of vit

value of the egg as human food,

TABLE 56

REQUIREMENTS OF POULTRY FOR VITAMIN A, ACCORDING TO VARIOUS AUTHORITIES

	Life stage	i u /kg diet	Form of vitamin	Authority	Comments
Chickens	Growing chicks	1100–2200	Vit A or cart	Castano *et al*	No safety margin
	Starting chicks	4400	Vit A or cart	N R C	With safety margin
	Growing chicks	4400	"	"	
	Laying hens	7300	"	"	
	Breeding hens	7300	"	"	
	Growth, laying and hatching	10 000	Vit A	Levy *et al*	
		20 000	Carotene		
Turkeys	Growing poults	2200	Vit A acetate	Gurcay *et al*	
		4400	Vit A as C L O		
		8400	Cart		
	Growing poults	3000	Vit A	Van Reen *et al*	For max growth
		5000	"		For normal health
	Growing poults	4000	Crypt	Scott *et al*	
	Growing poults	6000	Cart	Wilgus	
	Starting poults	8800	Vit. A or cart	N R C	
	Growing poults	8800	"	"	
	Growing poults	17,000	Carotene	Hinshaw *et al*	
	Young turkeys	17,000	Vit A	Wharton *et al*	Max growth

meet a difficulty similar to that mentioned in regard to milk Thus only a small fraction of any vitamin which is added to the diet will find its way into the egg We may suspect, however, that the absorption from the intestines of large amounts of vitamin A or provitamins is more efficient in hens than in bovines The fraction of the ingested vitamin which is transferred to the egg, therefore, may be somewhat greater than that which is transferred to the milk

Vitamin A and Parasitic Infection

Our knowledge of possible relationships between the vitamin A status and parasitism is both incomplete and uncertain, but scattered observations over a long period have suggested that the subject should repay further study The investigations which have so far been undertaken may be divided into two groups according to whether they have been concerned with the effect of the vitamin A status on parasitic infection or *vice versa* In view of the small number of communications in the whole field it will be convenient to deal in this chapter not only with parasitism in farm animals, but also with relevant experiments made upon small laboratory animals

The liability to parasitism in vitamin A deficiency

In a study of the prophylactic action of vitamin A in helminthiasis, Clapham[132] found that the vitamin A content of the diet had no effect on the course of infestations by *Heterakis gallinae* in chickens In albino rats, however, the intensity of infestation with *Parascaris equorum* was increased by deficiency of vitamin A Thus in deficient rats larger numbers of larvae hatched and migrated, and the rate of development was more rapid, than in rats supplied with the vitamin Clapham concluded from his different findings for these two parasites that the vitamin A status only affects the course of infestation when the parasite comes into close contact with the tissues of the host Later the same worker[133] studied the effect of vitamin A deficiency in pigs on resistance to infestation by *Ascaris lumbricoides* In general the deficient animals were less heavily infested than the controls.

Watt *et al*[134] studied the resistance of rats to infestation by *Nippastrongylus muris* When carefully counted numbers of larvae were injected subcutaneously slightly fewer worms were found later in the intestines of deficient animals than in those of controls dosed with vitamin A In other groups active immunisation was first promoted by repeated injections of larvae, followed by prolonged periods of subsistence with or without vitamin A After a later injection with more larvae many more worms were found in the intestines of the deficient animals than in those of the controls, but the reverse effect was found in the lungs In order to study passive

References p 494

immunisation rats were given simultaneous injections of larvae and of serum from infested rats In the intestines more worms were found in rats which had received serum from deficient donors than in those which had received serum from dosed donors Again the effect was reversed in the lungs

Another investigation on the rat, by Hitchcock and Bell[135], concerned the theory of Fibiger[136] that cancer may arise in the fore-stomachs of rats from infestation with the small nematode worm *Gangylomena neoplasticum* (*Spiroptera neoplasticum*), which has the cockroach as its intermediate host It was found that papilloma, down growths, squamous hyperplasia and hyperkeratosis occurred in animals which were either infested with the parasite or deficient in vitamin A When both infestation and deficiency were superimposed, however, the lesions were much intensified Fibiger received a Nobel award for his contribution to cancer research, but in the experience of Hitchcock and Bell the growths are never malignant

It is hardly necessary to comment that many of the findings which are reviewed in this section, apart from those of Hitchcock and Bell, seem contradictory and difficult to explain It seems probable that the factors which determine the effect of the nutritional status on parasitic infestation are complex We may guess that a well nourished host will support well nourished parasites On the other hand the migration of parasites or of their larvae, may be facilitated by defective barriers in the deficient animal

The effect of parasites on the vitamin A status

The most clear-cut evidence that parasitism can profoundly affect the vitamin A status has been reported by Davies[137] in relation to avian coccidiosis His first observations were made during routine examinations on 39 chickens from different parts of Britain The cases include infections with both the caecal type of the parasite (*Eimeria tenella*) and the intestinal type (*Eimeria necatrix*) Very low liver reserves of 0–126 i u per g were found, with a mean of only 6 9 i u Later an opportunity became available to compare the reserves of affected and unaffected birds from the same flock The food was a nutritionally balanced ration and contained 7700 i u of carotene per kg in the form of dried grass meal The liver reserves for groups of 10 birds each, of ages 8–24 weeks, were as follows

	Vitamin A i u /g liver Range	Average
Birds with coccidiosis	2–38	20 8
Unaffected birds	200–420	292

In sheep Eveloth *et al*[138] have made preliminary studies on the effect of infestation with filariform larvae on the conversion of carotene after de-

privation of vitamin A Better conversion was observed in one non infested sheep than in three which were infested Soliman[139] found low reserves of vitamin A as far as could be judged without the examination of control animals in the livers of 31 cattle which were infected by lung worms In experiments on guinea pigs animals which had been infected with *D filaria* were found 8 months later to have reserves averaging 2 5 i u per g, as compared with 23 i u in control animals which had not been infected Animals infected with *D viviparus* also had lower reserves than control animals

Supplies of Vitamin A for Farm Animals

The main sources of vitamin for farm stock may be divided up under three headings (1) Carotene may be provided by direct access to pasture, or by giving various forms of preserved herbage (2) Cryptoxanthin may be provided as yellow maize (3) Preformed vitamin A may be supplied as cod-liver oil or as some form of concentrate Each of these sources has special advantages and disadvantages which must be considered by the farmer in relation to both the health of the animals and the quality of its flesh milk or eggs as human food Thus when carotene is supplied to hens as dried grass meal the accompanying xanthophyll will give strong yellow colour to the egg yolk which is an advantage but also to the body fat, which may be a disadvantage in birds intended for roasting Yellow maize cannot be regarded as a rich source of the vitamin but its cryptoxanthin will have the virtue of stability Cod liver oil may be prized for containing vitamin D in addition to vitamin A but its excessive use may cause a fishy flavour in bacon poultry meat or eggs

Coates[140] gave the following values for three typical sources of carotene, cryptoxanthin and preformed vitamin A respectively

	i u per g
Dried grass meal or alfalfa (lucerne)	333–666
Maize	13 3
Cod liver oil (in Britain 1949)	400–800

Carotene in fresh and preserved herbage As might be expected there is general agreement that carotene is more concentrated in young, rapidly growing herbage than in mature fibrous herbage[141, 144]

The author s colleague, Dr V H Booth[145] found an average of 195 i u per g of fresh weight for 46 samples of pasture grass and 230 i u per g for 37 samples of clover Treatment of land with manure appears to increase

References p 494

the carotene contents of herbage in proportion to its effect in encouraging the production of young green foliage [141, 146]

The factors which promote the destruction of carotene in herbage have been studied by many investigators, and have been reviewed comprehensively in a book by Booth [147] Exposure to oxygen is the prime cause of destruction but heat, light moisture, enzymes and the state of subdivision of the material are also concerned As a general principle less carotene tends to be lost during rapid drying at a high temperature than during

In British drying machines the chopped

100° C, or a little more, for 45 minutes

In American machines the temperature is even higher, at about 500° C, and the time of treatment is reduced to about 30 seconds According to Waite and Sastry [148] the carotene losses during efficiently conducted drying and milling processes can be kept under 10% During storage the preservation of carotene in the dried meal depends mainly on the degree to which oxygen can be excluded, but the temperature and moisture content are also important In meal which was stored for 27 weeks Booth found losses of carotene of from 7 5 to 42 6%, according to whether the container was made of paper or polythene, whether the meal was moist or dry, whether the container was on the outside or inside of a pile and whether the meal was taken from the surface or the centre of the container The stability of carotene during storage may be increased by spraying the meal with an oily solution of a suitable antioxidant For this purpose Booth recommends 1 2 dihydro 6 ethoxy 2 2 4 trimethylquinoline

Hay making by time-honoured methods is cheaper than machine drying but the loss of carotene is much greater Under ideal conditions with a warm wind but little sunshine, as much as 50% of the original carotene may be preserved If the drying occupies a week of alternating rain and sunshine, however the amount of the provitamin which survives may be very small Underwood *et al* [149] found that carotene contents of oat hay after different periods of storage were as follows

Weeks storage	0	3	7	15
Carotene i u /g	44	24	15	7 5

In spite of the heavy losses of carotene involved in making and storing hay it must be remembered that this fodder is given to animals as a main component of their diet, and that it is not intended for use as a vitamin concentrate Well made hay will usually provide animals with all the carotene they need Attemps to preserve the vitamin may sometimes be unnecessary and uneconomic

The conditions affecting the stability of carotene in silage have been

extensively studied by Scharrer and Raker [150] The efficiency of preservation of the pigment in either lucerne or sugar beet tops was favoured by A I V solution (sulphuric and hydrochloric acids) and sometimes by molasses and other agents With lucerne treated with A I V solution and kept in a silo made of wood the preservation of carotene after 4 months of storage averaged 78% for the whole silo with variations between 65 and 89% between outer and inner layers When the A I V solution was replaced by molasses the average preservation was only 55% with a variation between layers of 21 to 97%

Sources of preformed vitamin A

For many years cod liver oil was the most important source of vitamin A for farm animals but its use has always been attended by certain risks Thus as long ago as 1924 Fridericia [151] observed the effect of rancid fats in destroying the vitamin The practical problem of retaining preformed vitamin A and also its provitamins in mixed foods has been studied intensively ever since [119, 152, 181] A detailed review by Ewing [182] has covered the effects of rancidity minerals charcoal antioxidants gelatin and fish and meat scraps with special reference to poultry foods In the experience of Templeton and Dudley [175] vitamin A deficiency occurred in chickens which received a mash which had been fortified when fresh with about 8000 i u of vitamin A per kg but which had become rancid on standing It is unfortunate that the addition to the mash of essential minerals such as manganese for the prevention of perosis in poultry may greatly accelerate the destruction of vitamin A

Recently an ingenious new method for the preservation of vitamin A in foods has been studied by Halverson and Hendrick [183] A concentrated source of the vitamin is first absorbed on a carrier with good antioxidant properties such as a mixture of defatted wheat germ and soya bean meals The meal is then moulded by a commercial process into minute pellets which are coated over with wax By this method the vitamin can be mixed evenly throughout the bulk of the food so that each mouthful contains the same amounts but with the prevention of actual contact between the food and the vitamin by the protective wax In storage tests food fortified with vitamin A in this way was found after 5 months to have retained 66% of its original potency as compared to 7–38% when other methods of fortification were employed

References p 494

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CHAPTER 35

Vitamin A and Sex

I. Influence of Sex on Vitamin A Requirements, Storage and Distribution

Our knowledge of interrelationships between vitamin A and sex has accumulated over a long period of time through several distinct channels Thus both clinical observations and animal experiments have suggested that males are more susceptible than females to the effects of vitamin A deficiency. In animals further evidence of sexual differences has been obtained in investigations on the efficiency of storage and distribution of the vitamin in the body, while similar distinctions have been reported between the levels of vitamin A and carotene in the blood of men and women under ordinary conditions of nutrition In yet another direction there have been intensive studies, which will be described in Chapter 36, concerning the influence of the vitamin on the condition and functioning of the sex organs. Evidence of some degree of interaction between vitamin A and the sex hormones will also be discussed

The Influence of Sex on the Sensitivity to Vitamin A Deficiency, and on Requirements for Vitamin A

Xerophthalmia and hemeralopia in humans

After the first world war an epidemic of night blindness, often associated by xerophthalmia, occurred in Vienna Birnbacher[1] later produced statistics on the outbreak, which he ascribed to deficiency of vitamin A He was particularly impressed by the much greater frequency of the symptoms in men than in women Thus out of 330 cases only 38 or 11.5%, were women or girls Fig 39 is based on Birnbacher's data for the relative numbers of males and females in different age groups who had suffered from hemeralopia In other investigations it was found that pregnancy tends to provoke hemeralopia, so two cases in which the women were pregnant were omitted from the female group It will be seen that the disparity between the sexes was greatest between the ages of 15–25 years, when 105 men were affected as compared with only two women Birnbacher

References p 512

pointed out that the same extreme disparity between the sexes had not been observed by Bloch and others in the incidence of xerophthalmia in infants (Chap 31) In his own investigation, however, there were 17 boys among 22 cases of hemeralopia in the age group of 10 years and under The observation of a sexual difference in such young children may help to answer a possible criticism that the greater liability of the male sex to hemeralopia could result from the nature of their employment or habits, rather than from a primary sexual difference in the metabolism of the vitamin

In commenting on these interesting findings Abels[2] and Poulsson[3] suggested that the superiority of the non pregnant woman over man in resisting night blindness reflected more efficient storage, which was made as provision against the demands during reproduction The difference between women and men, according to their views, was to be found in the greater thickness of the woman's fat deposits which also conferred a higher resistance to cold Thus Poulsson pointed to the hardihood of women in standing up to the rigours of sea bathing, and to their successes in channel swimming It would be unwise, of course, to accept the conclusion that women are superior to men in these directions without critical appraisal of the evidence Abels also directed attention to the greater liability of men to the effects of vitamin D deficiency At all ages, according to his state-

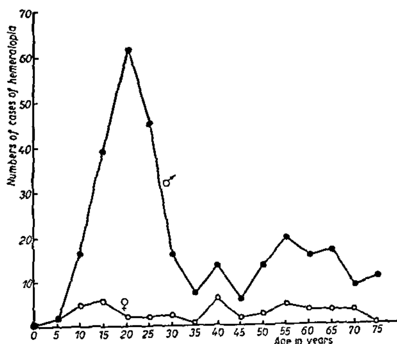


Fig 39 Hemeralopia in Vienna during the years 1919–1924 The graphs show the total numbers of cases observed arranged according to age *Upper graph* = males *Lower graph* = females (Birnbacher, 1928)

ments twice as many males as females suffer from rickets while late rickets is 15 times more frequent in adolescent boys than in girls

The vitamin A requirements of male and female rats

Soon after the publication of Birnbacher s report Coward and her colleagues [4] discussed the relative requirements of rats for vitamin A in the course of a paper which was mainly concerned with the suitability of various forms of casein for vitamin tests When high doses of cod liver oil were given to young rats which had been made deficient in vitamin A the males grew more rapidly than the females as might well be expected from their much greater weight at maturity At low doses however the relationship was reversed This suggested that the male requires a larger dose to ensure survival than the female a conclusion which was confirmed by comparisons of the incidence of fatalities among males and females in a long series of tests In returning to the same problem 11 years later Coward [5] produced a large accumulation of data on rats which had been used for tests of various sources of vitamin A and carotene Statistical examination of the figures left no doubt that the greater mortality rate in males was highly significant Coward suggested that her findings might indicate one of the causes for the slightly greater mortality among male human infants than among females during the first year of life

Later Mayer and Krehl [6] claimed that male rats deprived of vitamin A showed signs of deficiency before the females were affected According to Booth [7] however this effect is not important In his own experience male rats show signs of deficiency at the same time as females or only slightly sooner

THE INFLUENCE OF SEX ON THE STORAGE AND DISTRIBUTION OF VITAMIN A

Liver reserves of vitamin A in cows and steers

Poulsson [3] had commented on the influence of sex on the subcutaneous fat deposits in bovines In cows he found the fat to be more plentiful softer and yellower than in bulls of the same age Moreover in the antimony trichloride reaction the colour given by the cow s fat was 15 times more intense than that given by bull s fat

Ender [8] followed up this lead in a comparison of the concentrations of the vitamin in the livers of cows and bulls Very variable results were found in both groups but the difference between the mean values was striking Thus for a group of 48 cows an average of 71 Lovibond blue units per g of liver was found as compared to only 15 blue units for a group of 81 bulls The superiority of the cow s liver over that of the bull was found to hold

References p 512

good when the groups were sub-divided according to age, but no assurance was given that the dietary histories of the two sexes were similar

In groups of 11 cow and 15 bull calves of ages up to 10 weeks the individual values were again very variable, but the means of 11 6 and 9 7 blue units per g were not significantly different

Liver reserves of vitamin A in rats In an investigation with rats on the interrelationship between vitamin A and thyroxine Logaras and Drummond [9] found that in all their groups the concentrations of vitamin A in the livers of females tended to be somewhat greater than in males No comment was made by the authors on this finding, but Kimble [10] drew attention to its significance in relation to her own studies on human blood (Chap 29) In a paper directly concerned with the influence of sex on vitamin A metabolism Bult and Sorgdrager [11] found that when rats were restricted to a diet deficient in vitamin A the liver reserves of females were depleted less rapidly than those of males, with depletion in castrated males at an intermediate rate

Many other investigators have subsequently placed the superiority of the female liver reserves beyond doubt under widely different dietary conditions Thus Brenner, Brookes and Roberts [12] found that when rats were given massive doses of vitamin A for 5 weeks the stores accumulated in the females were higher than in the males, and were dispersed less rapidly during a subsequent period of depletion Smaller doses of either preformed vitamin A or carotene were administered by Esh and Sutton [13] in the course of a study mainly concerned with the effect of lecithin on their absorption After rats had been depleted, and then dosed daily with either 17 1 u of preformed vitamin A or 33 1 g of carotene the liver reserves of the animals not given lecithin were as follows

	No of rats	Total liver stores of vitamin A i u	
		Males	Females
Dosed with vitamin A	16	48	68
Dosed with carotene	12	150	216

The differences between the sexes were statistically significant, and were equally pronounced in other groups which were given lecithin or choline in addition to vitamin A or carotene

Booth [7] has found ratios of 1 2–2 0 between the liver stores of male and female rats dosed either with preformed vitamin A or carotene The exact ratio appeared to depend on the period of dosing In rats given carotene in

various forms no difference could be detected between the sexes in the percentages of the ingested dose which were excreted in the faeces

Against this array of evidence Lemley, Brown, Bird and Emmett [14] were unable to find any consistent effect of sex on the storage of vitamin A in rats given a wide range of doses Moore, Sharman and Ward [15] found that female rats stored more vitamin A in their livers and kidneys combined than males at low levels of dosing, but that the difference became less pronounced when massive doses were given The results of Callison and Knowles [16] supported the conclusion that greater liver reserves are accumulated in females than in males given equal doses per kg of body weight When rats which had been allowed to accumulate reserves were given a deficient diet, however sex was found to have no influence on the development of signs of deficiency, in the form of hemeralopia or xerophthalmia. The magnitude of the small reserves of vitamin remaining in the liver when these signs were observed was also unaffected by sex

Vitamin A in the blood of men and women As a demonstration of the application of her method for the estimation of carotene and vitamin A in human blood plasma Kimble [10] presented data for 30 normal men and 34 normal women Her average values were as follows

	Men	*Women*
Carotene (μg per 100 ml)	166	187
Vitamin A (i u per 100 ml)	127	91

Similar differences have been found by Murrill *et al* [17], Highman [18], Moore and Leitner [19], Campbell and Tonks [20], Hoffmann, Schneider and Quamo [21] and Lahiri and Scandrett [22] A higher average for vitamin A in males than in females, but not a lower carotene, was reported by Abels *et al* [23] In all these investigations, it must be realised the values found for vitamin A and carotene in each sex covered wide overlapping ranges which made it impossible to observe any consistent differences between individual men and women The averages for large groups, however, seem to have proved the influence of sex beyond any reasonable doubt (Figs 40 and 41)

Human blood after dosing with vitamin A According to the interesting observations of Week and Sevigne [24] the general superiority of the man's blood over woman's in its concentration of vitamin A is not only shown in specimens collected under ordinary dietary conditions but also after massive dosing In Fig 42, which is based upon their results, the mean levels of vitamin A reached in the blood of 18 men at intervals after the ingestion of a single dose of 450 000 i u of vitamin A alcohol are compared with the corresponding means for 7 women It will

References p 512

Fig 40. Vitamin A in the blood plasma of men and women Broken line = men Solid line = women. Values found for 324 men and 219 women during the years 1952–1955 have been divided up according to arbitrary ranges of magnitude, and the percentages of cases falling within each range have been calculated for each

Fig 41 Carotenoids in the blood plasma of men and women Broken line = men Solid line = women The graphs were obtained in the same way, and for the same subjects, as in Fig 40 (Leitner, Moore and Sharman, unpublished data).

be seen that the levels attained in the men were much greater than in the women at all times after dosing with a maximum after 5 hours which was nearly double the maximum reached in the women

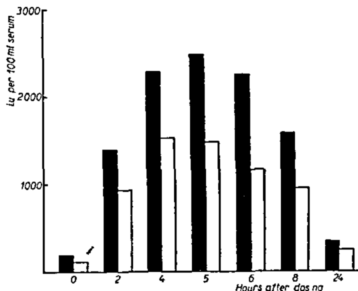


Fig 42 Vitamin A in the blood plasma of men and women at different times after a single dose of 450 000 i u of vitamin A alcohol Black columns = averages for 18 men White columns — averages for 7 women (Week and Sevigne 1950)

Animals blood Information on the influence of sex on the blood levels of vitamin A in experimental and farm animals is less adequate In their rats which were allowed to accumulate large reserves of vitamin A before restriction to a deficient diet Brenner *et al* [12] generally observed higher levels in the blood of males than of females The experience of Moore Sharman and Ward [15] with rats killed after various doses of vitamin A was similar Male and female rats which had been dosed with 320 i u of vitamin A daily had levels of 98 and 76 i u respectively in their blood plasma which were in a reverse relationship to the concentrations of 435 and 755 i u per g found in their livers At most other levels of dosing the blood of the males also contained more vitamin A than that of the females, but the dosage of 320 i u perhaps deserves special attention in having caused the accumulation of reserves of the order to be expected in wild rats

In regard to bovines an early report by Lundborg [25] claimed that the blood of cows contained about three times as much vitamin A as that of steers but this clue does not seem to have been followed up

Menstruation Within the female sex there is evidence that the level of vitamin A in the blood may be influenced by the activities of the ovaries Thus Laurence and Sobel [26] have reported cyclical

References p 512

changes during the menstrual cycle (Fig 43) The lowest level is usually found at about the onset of menstruation, after which there is a fairly steady rise to a peak at the 17th day The fall before the next period is interrupted by a further peak on the 26th day These observations gain added interest when contrasted with findings by Oliver and Boyd [27] for other constituents of the plasma lipids Thus free cholesterol varies little throughout the cycle, but esterified cholesterol shows considerable variations The lowest points for esterified cholesterol, and also for phospholipids, seem to be on the first and 14th day of the cycle, with maxima on about the 7th and 21st days As far as can be gathered from the data at present available, therefore, the curve for vitamin A is not merely a reflection of a general curve for all the plasma lipids

The fall of vitamin A in the blood plasma during pregnancy, and its rise after parturition, has already been discussed in Chapter 21

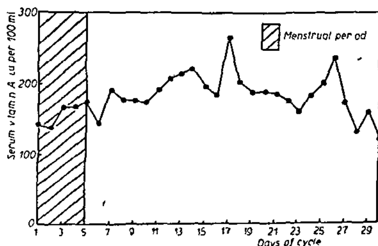


Fig 43 The menstrual cycle A composite graph made from data for the fasting level of vitamin A in the serum of six healthy women in whom a total of 14 cycles were studied In plotting the graph all the cycles were equalised to 30 days which was the average length observed throughout the investigation (Laurence and Sobel 1953)

Distribution of vitamin A between the liver and kidneys Except for the evidence of the wide variations between the vitamin A contents of the livers of cows and steers, in which dietary differences may have complicated the effect of sex, the margin between male and female values in the work so far described has usually been comparatively small The concentrations of vitamin A in the livers of female rats for example, have exceeded those in male rats by some 50% Man's blood has contained 20–30% more vitamin A than woman's blood and 10–15% less carotene These differences are not far removed from ranges in which they might reflect known or unsuspected differences in the major components of the blood and tissues, such as fat and protein

It therefore seems important that under experimental conditions in the rat the effects of sex on the distribution of the vitamin may be greatly emphasised Thus in confirming the observation of Johnson and Baumann [28], that at low levels of dosing the kidney accumulates higher concentrations than the liver Moore and Sharman [29] noticed that sex had a pronounced influence *In rats which were dosed with 40 i u of vitamin A daily the* superiority of the liver reserves in the female was substantiated with an average of 24 4 i u against only 9 4 i u per g in the male In the kidney, however the relationship was completely reversed with 24 1 i u per g in the male as against only 4 9 i u in the female The results for the individual animals in this experiment are shown in Fig 44

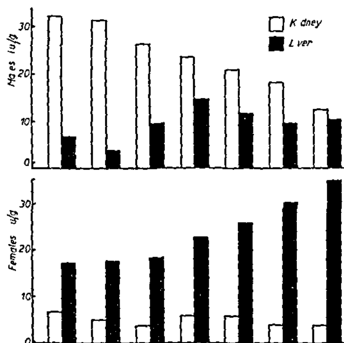


Fig 44 Vitamin A in the livers and kidneys of rats which were dosed with 40 i u daily Above = males Below = females Note that in the males but not in the females the concentrations of vitamin in the kidney regularly exceeded those in the liver (Moore and Sharman 1950)

Such striking differences between the sexes it must be emphasised can only be observed at carefully chosen levels of dosing When the allowance of vitamin A is raised the disparity between the concentrations in the male and female livers is reduced and the level in the male kidneys shows a paradoxical decrease to about the level found in the female at the lower dose (Fig 45) From the data of Moore Sharman and Ward [15] we may compare the distributions after daily doses of 40 and 320 i u

References p 512

changes during the menstrual cycle (Fig 43) The lowest level is usually found at about the onset of menstruation after which there is a fairly steady rise to a peak at the 17th day The fall before the next period is interrupted by a further peak on the 26th day These observations gain added interest when contrasted with findings by Oliver and Boyd [27] for other constituents of the plasma lipids Thus free cholesterol varies little throughout the cycle but esterified cholesterol shows considerable variations The lowest points for esterified cholesterol and also for phospholipids seem to be on the first and 14th day of the cycle with maxima on about the 7th and 21st days As far as can be gathered from the data at present available therefore the curve for vitamin A is not merely a reflection of a general curve for all the plasma lipids

The fall of *vitamin A in the blood plasma during pregnancy* and its rise after parturition, has already been discussed in Chapter 21

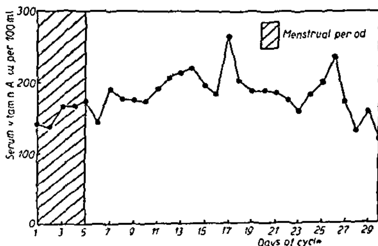


Fig 43 The menstrual cycle A composite graph made from data for the fasting level of vitamin A in the serum of six healthy women in whom a total of 14 cycles were studied In plotting the graph all the cycles were equalised to 30 days which was the average length observed throughout the investigation (Laurence and Sobel 1953)

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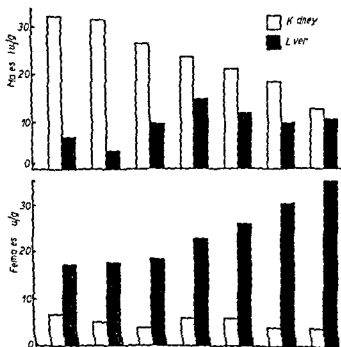


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Daily dose	No of rats		Mean vitamin A i u /g			
			Liver		Kidneys	
	Male	Female	M	F	M	F
40 i u	12	7	10 4	24 4	37 5	4 9
320 i u	11	6	435	755	8 2	5 7

The values given above for the 40 i u. level of dosing include the data on which Fig 44 was based, but supplemented by the results of extra experiments on males The remarkable fall in the concentration of vitamin in the kidney of the male from 37 5 i u at the lower dose rate to 8 2 i u at the higher dose rate is emphasised

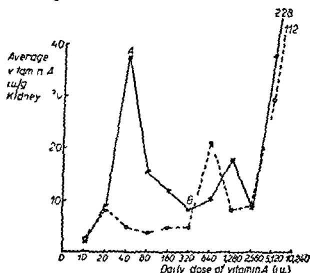


Fig 45 Vitamin A in the kidneys of groups of rats as influenced by the magnitude of the daily dose Males = solid line with crosses Females = broken line with circles The paradoxical positions of points A and B were repeatedly confirmed but the fluctuations at dosages of 640–2560 i u were observed only in single experiments (Moore Sharman and Ward 1951)

The Effects of Castration or Treatment with Hormones on the Distribution of Vitamin A in the Blood and Tissues

Castration Booth[7] confirmed the finding of Bult and Sorgdrager[11] that castrated male rats lose their vitamin A reserves rather less rapidly than entire males He also found that ovariectomised females lost their reserves rather more slowly than entire females In mice Rush, Truscott and Rush[30] found that castration increased the reserves accumulated when the animals were kept for long periods upon a diet rich in the vitamin

Treatment with sex hormones For experiments on the administration of hormones Chapman *et al*[31] used immature pullets They found that when injections of oestradiol were given on six

alternate days, with injection of testosterone on the remaining days the level of vitamin A finally attained in the blood plasma could be correlated with the dose of oestrogen Thus with no oestradiol the blood serum averaged 136 i u of vitamin A per ml with doses of 6 mg the level was 226 i u, with 12 mg 410 i u and with 24 mg 565 i u Parallel increases were observed in the calcium content of the serum In human subjects Danish and Klopp [32] obtained somewhat similar results in finding that injections of either testosterone or stilboestrol increased the level of vitamin A in the blood Little or no effect however was observed by Williamson [33] when six weeks old rabbits were injected with oestradiol

Effect of sex hormones on vitamin A in the kidney

The accumulation of vitamin A in the kidney at low levels of dosing, which has been discussed in the preceding paragraph suggested to Moore, Sharman and Ward [34] another means of studying the effects of castration and injections of hormones into male rats The animals were allowed 40 i u of vitamin A daily for about 4 weeks, which was calculated in entire animals to cause the concentration of vitamin A in the kidneys in preference to the liver Table 57 shows the results which were obtained

TABLE 57

AVERAGE VITAMIN A CONTENTS OF THE KIDNEYS AND LIVERS OF RATS GIVEN 40 I U OF VITAMIN A DAILY FOR 27–29 DAYS (MOORE *et al* 1951)

Treatment	Mean wt increase (g)	Wt of seminal vesicles (g)	Vitamin A in Kidneys Total i u	Kidneys i u/g	Vitamin A in Liver Total i u	Liver i u/g
Not castrated	109	0 575	75 0	37 4	118	10 5
Castrated no injection	98	0 004	71 9	42 7	230	26 0
Castrated + testosterone	97	0 705	76 1	35 7	183	19 9
Castrated + testosterone with limited food	40	0 575	25 7	18 7	250	37 6
Castrated + oestradiol	40	0 064	7 5	4 6	261	35 2

The data for the livers will be seen to be in agreement with Truscott's finding that castration increases the storage of vitamin A This increase was shown in varying degrees even with injections of sex hormones In the kidneys the concentration of vitamin A was little removed from the mean of 37 4 i u per g found for the entire animals either by castration, or by castration combined by injections of testosterone Castration combined with injections of oestradiol however, reduced the concentration of vitamin in the kidneys to a level of 4 6 i u per g typical of females When castrated animals were given injections of testosterone but were limited in

References p 512

their food intake so as to grow no more than the animals injected with oestradiol, the concentration of vitamin A in their kidneys was still in the male range at 18 7 i u per g

For some unexplained reason attempts to increase the concentration of vitamin A in the kidneys of female rats by ovariectomy proved unsuccessful, even when injections of testosterone were given [34] The levels of vitamin A in the kidneys of sexually immature rats of either sex varied between the limits for adult males and adult females but were reduced to levels typical of mature females by injections of oestradiol [36]

The Interplay of Sex Growth and Other Factors in Determining the Distribution of Vitamin A

Growth In experiments on the effects of growth and the metabolic rate on the expenditure of vitamin A, Johnson and Baumann [37] restricted rats with initial total reserves of about 600 i u to various diets deficient in vitamin A When the diet was otherwise adequate in quantity and quality they observed, without examining the effect of sex, that the fall of vitamin A in the liver was accompanied by a 10–20 fold increase in the kidneys When the growth of the animals was suppressed by any means, such as by allowing inadequate amounts of the diet, or by withholding tryptophan or aneurin the expenditure of the liver reserves of vitamin A was retarded and the transfer of vitamin to the kidneys almost completely suppressed

Booth [7] followed up this clue When young male and female rats were given single doses of vitamin A and were killed soon afterwards the levels

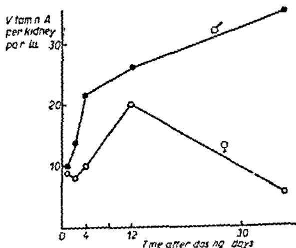


Fig 46 Vitamin A in the kidneys of male and female rats at various times after single doses of 480–1250 i u of vitamin A Upper graph = males Lower graph = females The data were collected in several experiments in which 238 animals were used (Booth 195?)

of vitamin in the kidneys were low in both sexes In male animals killed after longer intervals however, the level of vitamin in the kidneys rose consistently, whereas in the females there was only a temporary rise in 12 days after dosing (Fig 46)

The close relationships between growth sex and vitamin A make it difficult to decide whether the differences in the distribution of vitamin A between male and female animals are a primary effect of the sex hormones or are secondary to their general effects including modifications of the growth rate As arguments against the view that the growth rate is the determining factor at least in deciding the level of vitamin in the kidney we may point to the low concentrations of vitamin which are usually found in the kidneys of females growing at the maximum rate for their sex In males moreover we have seen that an increase in the allowance of vitamin A may decrease the concentration of vitamin in the kidneys although there will be no adverse effect on the growth rate (see page 486) Booth [7] found that rapidly growing female rats from small litters stored vitamin A in their livers more efficiently than slowly growing males from larger litters This is the reverse of what might be expected on the theory that growth *per se* causes a migration of vitamin A to the kidneys at the expense of the liver reserves

The influence of sex hormones on the liver and kidneys

At the present stage of our knowledge oestradiol appears to affect the distribution of vitamin A much more drastically than testosterone and it is noteworthy that it both prevents the appearance of vitamin A in the rat s kidney and inhibits growth On the other hand ovariectomy, which often causes an increased rate of growth, does not seem to increase the concentration of the vitamin in the kidneys

As a working hypothesis it may be useful to assume that when the liver is low in vitamin A it exhibits a readily reversible defect in its power to hold the vitamin This defect it appears may be overcome either by increasing the intake of vitamin above the necessary threshold or by giving oestradiol Evidence of the influence of oestrogens in affecting the liver is certainly not lacking Thus Gyorgy [38, 39] found that oestrogens have lipotrophic activity, while several other workers have found that they considerably increase the liver weight of birds [40–42] A puzzling point is raised by the observation of Chapman *et al* [31] that oestradiol greatly increases the level of vitamin A in the blood of pullets, no corresponding increase has been noticed in the rat

Attention may also be drawn to the interesting observations of Crabtree [43] that the histological structure of the kidneys of mice is influenced by sex Thus in males the percentage of the Bowman's capsules in which the parietal

References p 512

layer is covered with cuboidal cells is higher than in females Even if similar differences were proved to occur in the kidneys of rats, however, it is doubtful whether they could account for the divergences in the concentration of vitamin A Castration was found by Crabtree [44] to reduce the number of capsules covered with cuboidal cells in the kidneys of her mice, but Moore, Sharman and Ward [34] found that it had little effect on the concentration of vitamin A in the kidneys It is also well known, of course, that injections of testosterone often tend to increase the size of the kidneys

The influence of sex on fat metabolism. Readers will be aware, of course, that sex is an important factor in determining the rate of incidence of certain common diseases. For example men are much more liable than women to suffer from coronary artery disease Throughout the field of biochemistry, moreover, there have been numerous instances in which sexual differences have been clearly established A detailed review of this fascinating subject is beyond the scope of this book, but a passing reference to Deuel's interesting observations on sexual differences in carbohydrate metabolism seems appropriate Thus during starvation men were found to be more resistant than women to ketosis [45] and the same difference was later confirmed between male and female rats which had received diets high in fat [46] The livers of male rats tended to contain more glycogen than those of females, and the level of the hepatic fat declined less rapidly during starvation. It would be interesting to explore the possibility that the sexual differences in vitamin A metabolism, described in this chapter, may form part of the same picture as those established by Deuel in regard to carbohydrate and fat metabolism

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CHAPTER 36

Vitamin A and Sex

II. Influence of Vitamin A on the Condition and Functioning of the Sex Organs

In the preceding chapter we have discussed the effect of sex on the requirement of the animal for vitamin A, and on its storage and distribution in the body We must now turn our attention to the reverse side of the picture, and review the many investigations which have been made during the last 30 years on the effect of vitamin A, and its deficiency, on the condition and functioning of the sex organs.

VITAMIN A DEFICIENCY AND THE MALE SEX ORGANS

Testes Early accounts of the atrophy of the testes in vitamin A deficiency were given by Gross [1] and by Wolbach and Howe [2] who found a reduction to about half the usual size in rats. Many investigators confirmed this finding in rats [3-8], mice [9] and guinea pigs [10] but according to some reports the atrophy is preceded by oedema. Mason [11] commented on the highly organised structure and the great metabolic activity of the germinal epithelium, which he accepted as the cause of its being injured in vitamin A deficiency earlier and more severely than other epithelial structures In the writer's experience the particular vulnerability of the testes to deficiency of the vitamin is illustrated by their remaining undersized in rats which have been exposed to vitamin A deficiency and then cured by doses of the vitamin sufficient to allow only moderate growth Mason has found, however, that the injuries caused to the testes by deficiency of vitamin A, unlike those caused by deficiency of vitamin E, may be eventually cured by prolonged treatment with adequate doses of the vitamin

Secondary male sex organs The extent to which vitamin A is concerned in the development of secondary male sexual characteristics, including the development and maintenance of the epididymes, seminal vesicles and prostate glands, has not been completely elucidated Mason found that these organs were atrophied in his vitamin A deficient rats He considered, however, that this atrophy was not a specific

effect of lack of the vitamin, but was merely a secondary outcome of the reductions in body growth, and the general standard of nutrition, which were incurred in its absence. Taking the matter further Mayer and Truant [12] found that when testosterone was injected into vitamin A deficient rats, both castrated and entire, their secondary sexual organs responded no less vigorously than in normally nourished animals This evidence suggested that vitamin A deficiency interferes with the ability of the testes to synthesise or release testosterone, but that the seminal vesicles, and other target areas, are able to respond when testosterone is supplied Further work by Mayer and Goddard [13], however, introduced a complication by showing that the gonadotrophic hormone of the anterior pituitary gland was no less effective than testosterone in stimulating the development of the secondary sexual organs of deficient rats The seminal vesicles, on this evidence, fail to develop because of a deficiency in the functioning of the testes, which in turn fail in their function on account of a pituitary abnormality.

Against this theory Mason had concluded that the lesion in the testicular epithelium is a primary effect of vitamin A deficiency, and not a secondary effect of a pituitary lesion With Wolfe [14] he had found that the pituitaries of vitamin A deficient rats retained their gonadotrophic power, at least as measured by the production of precocious sexual development in females. Sutton and Brief [15], moreover, found that in vitamin A deficiency the gonadotrophic activity of the pituitary was increased, as in castration. They regarded this increase, which was greater in males than in females, as a compensatory reaction to the primary injury in the gonads

In the writer's own experience atrophy of the testes in rats deficient in vitamin A is often associated with well developed seminal vesicles, which sometimes become infected with pyogenic organisms The disparity of this finding with those of other workers may depend on whether the rats are allowed to reach sexual maturity before the effects of the deficiency are incurred.

Vitamin A and the Female Sex Organs

Ovaries The ovaries have been much less intensively studied than the testes as the site of injuries resulting from deficiency of vitamin A Presumably the lesions sustained are both less characteristic in appearance and less physiologically important than those produced in the testes Truscott [16] has reported, however, that the ovaries of deficient rats are small in size Thus normal rats when 11 weeks old had body weights of 180 g and ovaries weighing 26 mg, as compared with 100 g and 5 6 mg in rats deficient in vitamin A. The possibility that vitamin A may play

References p 523

some part in the metabolism of the ovary, moreover, has been suggested by a fascinating study by Ragins and Popper [17] of the fluorescence seen in the ovaries in various phases of their activity In the examination of ovaries obtained at operation from women and children the greenish yellow fluorescence attributed to vitamin A was observed in the *corpora lutea* and *atretica*, in follicular cysts and in certain areas of the *stroma* Fluorescence was seen in the ovaries of a child only 5 months old, but ovaries taken from women after the climacteric did not fluoresce

The vaginal smear in vitamin A deficiency As already mentioned in Chapter 25 the uterus and vagina, in contrast to the ovaries show highly characteristic changes in vitamin A deficiency These abnormalities have been studied mainly in the rat by inspection of vaginal smears, which in the normal animal provide an indication of the cyclical changes in the conditions of the epithelium of the vagina and uterus

The normal oestrous cycles During the preparatory period preceding oestrus, or the condition commonly known as "heat", the lining of the vagina is made up of large nucleated cells which are capable of forming mucus Underneath lies another layer of cells which tend to become cornified, as in the top layer of the epidermis, with the formation of dry thin scales Heat ensues with the shedding of the nucleated cells and the emergence and the development of the cornified layer When this layer has in its turn been shed the cycle is completed by the development of another layer of nucleated cells

According to Long and Evans [18] the average total time of the oestrous cycle in the rat is 4 6 days Smears may be taken by the insertion into the vagina of a small spatula or glass rod, and may be examined microscopically under lower power (Chap 5, Fig 2) When they are taken from normally nourished animals, stringy mucus containing many leucocytes and also small irregularly shaped epithelial cells, which occur singly rather than in groups, are seen during a period lasting for about half the cycle This picture denotes the dioestrous interval during which the vagina is moist and pink, and sexual activity suppressed Stage one of oestrous, with the disappearance of leucocytes from the smear and the appearance of numerous round nucleated epithelial cells, which are uniform in size and often arranged together in sheets, is observed for the next 12 hours Stage two then suddenly intervenes with the replacement of the nucleated cells by the typical scales commonly known as "cornified cells" The mucous membrane of the vagina is now dry and white, and the animal is ready to accept coitus *Stage three* is an emphasis of stage two, but with the rat no longer willing to copulate, and the two stages together occupy 30 hours In stage four, which lasts only 6 hours, leucocytes and detached nucleated cells make their reap-

pearance until the complete replacement of the cornified cells brings back the dioestrous interval

Vaginal keratinisation in vitamin A deficiency

As early as 1922 Evans and Bishop[19] observed that in rats deficient in vitamin A the vaginal smear remained constantly in stages two to three of the cycle with cornified cells predominating This abnormality, which was later further studied by Evans[20], occurred regularly in all females exposed to deficiency even if they were castrated, and could be observed earlier than xerophthalmia or the formation of abscesses In spite of persistent vaginal cornification, however, animals which had not been castrated continued to ovulate and to form *corpora lutea*, either irregularly or at normal intervals, and could be induced to accept coitus at intervals Vaginal cornification under the stress of vitamin A deficiency, therefore occurred irrespective of the presence or absence of ovarian stimulation

The claim of Evans that the vaginal smear provided a clear indication of the onset of vitamin A deficiency was questioned by some workers[21–23] and supported by others[24–26] Aberle[27] invariably found cornified cells in the vaginal smears of deficient animals and in histological sections confirmed that the peripheral layer of the epithelium was cornified before being shed into the lumen Vaginal cornification always preceded other symptoms of avitaminosis, and the length of the depletion period before its appearance depended on the degree of purification of the basal diet and the initial vitamin A reserves of the rats Moreover when deficient rats were injected with a gonotrophic hormone, prepared by extracting human placentas with alcohol and ether, they failed to show the normal production of mucoid cells in the vagina[28]

Detailed studies by Mason and his colleagues[29, 30] amply confirmed that when rats are given a diet deficient in vitamin A vaginal cornification invariably precedes xerophthalmia in its appearance The times at which both abnormalities appeared were unaffected by removal of the ovaries From a study of some 52 000 smears it was concluded that the effects of vitamin A deficiency on the vaginal smear were first seen in a shortening of the pro oestrous phase (stage 1) and the prolonging of the phase of cornification (stages 2 and 3) up to several days The animals came on "heat" on the last day of this prolonged cornification At the same time there was a tendency for the substitution of cornified cells for the detached nucleated cells in the dioestrous phase, with the result that as deficiency advanced the smear appeared to be completely and continuously cornified

Even when dry stained smears indicated continual cornification, however, the persistence of cyclic changes could be demonstrated by supravital staining with neutral red, applied by lavage according to the method of

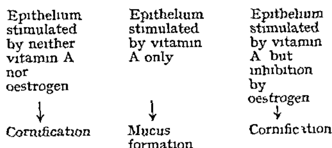
References p 523

Guttmacher [31] Mason concluded that the excessive keratinisation was caused directly by vitamin A deficiency, but that the prolongation and irregularity of the oestrous cycle were secondary effects of the associated *failure in* growth and subsequent decline in body weight

The balance between the formation of mucus and keratin

It has been mentioned that Aberle [28] found that in vitamin A deficiency the vagina failed to produce mucoid cells in response to injections of placental *extract Mason* [30] *soon followed* with a suggestion that in deficiency of vitamin A there is a defective formation of mucin made up of glyco proteins, in the epithelial cells The production of keratin made up of albuminoids, is correspondingly increased Vitamin A he suggested, may play an important part in protein metabolism, or synthesis, within the epithelial cell *It was impossible, however, to decide whether the* keratinising process in vitamin A deficiency differed in any way from that occurring at oestrus in normal rats under the action *of hormones*

Although the effects of vitamin A and oestrogens on the vaginal epithelium certainly appear to be opposed we cannot ascribe continuous vaginal *cornification to the uninhibited action of oestrogens in the absence of* vitamin A The occurrence of cornification in ovariectomised animals deficient in vitamin A obviously rules out this otherwise attractive interpretation Instead we may consider that the epithelium even in the absence of oestrogens, tends to become cornified unless it is stimulated to form mucus *by the action of vitamin A According to this conception cornification* during normal oestrus can be explained by interference in the action of vitamin A by the periodical accumulation of oestrogens These relationships may be summarised as follows



In the author s own experience, however, the ' mucus" which is found in the vaginal smears of castrated rats usually takes the form of large thin sheets These sheets which often crumple and fold up could give the impression that the animal is continuously in stage 3 of the oestrus cycle Perhaps it is too much to expect that smears from such animals in which the uterus has atrophied, should conform completely to the typical pictures

seen in dioestrus or stage 1, which presumably represent the phases opposite to oestrus in the uncastrated rat.

Dosage rate required for normal oestrous cycles

Kuhn and Brockmann [32] found that the appearance of cornified cells in the vaginal smears of vitamin A deficient rats could be checked by daily doses of 2 5 μg of β-carotene, which were also the minimum required to prevent death and restore growth For the prompt restoration of regular oestrous cycles, however, doses of 20 μg were necessary. It was pointed out that the same margin of about 8 times the minimal dose was also needed to cause the appearance of measurable stores of vitamin A in the liver. According to the mechanisms outlined in the preceding paragraph it is difficult, of course, to understand why less carotene was required to check cornification completely than to produce conditions under which it periodically recurs Mason's view that the period of the oestrus cycle depends on the weight and general condition of the animal may supply an explanation.

The restoration of normal smears in deficient rats after the administration of graded doses of cod-liver oil was studied by Mason and Ellison [30]. The doses needed to restore the oestrus cycle were not compared with the minimum requirements for growth and liver storage, but it was found that the delay in the re-establishment of the cycle was shorter with high doses than with lower doses The administration of vitamin A invariably caused the reappearance of mucus in the smear. In castrated animals the mucus persisted continuously as long as adequate doses of the vitamin were given.

The antagonism between vitamin A and oestrogens

The evidence which we have already reviewed has shown that vitamin A is essential for the formation of mucus by the vaginal epithelium, and that keratinisation may be induced by the withdrawal of vitamin A or by the activity of the ovaries in secreting oestrogen. It might be considered, however, that this apparent antagonism only amounted to an overlapping between two mechanisms Thus vitamin A might be considered as necessary for the formation of mucus and oestrogen for the normal promotion of the oestrus cycle Once both these requirements had been adequately satisfied no further antagonism between the two factors would be expected

It is interesting, therefore, that the opposition still seems to persist when doses above the usual levels are given Hohlweg [33] has found that when ovariectomised rats are given massive doses of vitamin A, sufficiently large to cause skin changes and loss of body weight, injections of oestradiol fail to cause the appearance of cornified cells in the vagina smear, which continue to show the mucus, leucocytes and small epithelial cells typical of dioestrus (Plate 46) Great excess of vitamin A, therefore, prevented the transition

References p 523

from dioestrus to oestrus In the reverse direction even more interesting results have been obtained by the administration of single doses of vitamin A to rats in which the vaginal smears had been made continuously cornified by injections of oestrogen A dose of 10 000 i u had no effect on the smear but doses of 30 000 or 60 000 i u caused the disappearance of cornified cells within 48 hours This effect could be demonstrated irrespective of the doses of oestrogen which were given and occurred with such regularity that it could be made the basis of a method for assaying vitamin A in rich concentrates[34] Carotene had no action in preventing oestrus

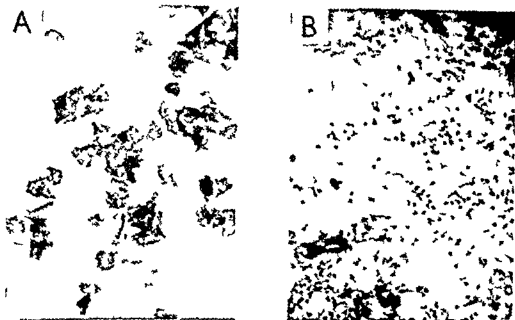


Plate 46 The action of massive dosing with vitamin A in preventing the response to an oestrogen in rats *A* The vaginal smear of a normally nourished ovariectomised rat after the injection of ? µg of dienoestrol diacetate Note the scales of keratin indicating response to the oestrogen *B* The smear of a rat which had been similarly treated except for oral dosing with 60 000 i u of vitamin A acetate daily for 12 days before the injection of the oestrogen Note the complete failure to respond The smear indicates the persistence of dioestrus with the presence of mucus and leucocytes

Topical treatment of the cornified vagina with vitamin A has also been investigated Thus Kahn and Bern[35] inserted pellets of α oestradiol into ovariectomised rats and kept them until their vaginal smears had become cornified They were then injected by the vagina for 2–16 days with daily doses of 1500–2500 i u of vitamin A dissolved in sesame oil 39 i u of vitamin A as cod liver oil or plain sesame oil After two or more days of treatment with the high doses of vitamin A but not with cod liver oil or sesame oils the vaginal smears showed definite changes with the appearance of ovoid and round cells including many with large vacuoles in their cytoplasm Later the effects of vitamin A and oestrogen were compared

on non sexual epithelium [36] The thickness of the epidermis in rats was about doubled after the application of vitamin A for 10 days The most prominent histological feature was an increase in the *stratum granulosum* which was attributed to either a stimulation of keratohyalin formation by the vitamin or to a decrease in the rate of keratin formation The effect was only local and untreated areas of skin remained unaffected Oestrogen neither affected the condition of the skin when administered by itself nor counteracted the stimulatory effect of the vitamin when administered simultaneously

VITAMIN A THERAPY IN DISEASES OF WOMEN

Senile vaginitis Experimental studies of the influence of vitamin A on the condition of the vagina have been supplemented by a few isolated clinical observations on women Simpson and Mason [37] tested the value of the vitamin in the treatment of senile vaginitis This condition known also as agglutinative or adhesive vaginitis chronic vaginitis or chronic non specific vaginitis occurs most commonly in elderly women either during or after the menopause but it is also seen in young women who have been castrated by radiation or surgery The patient most frequently complains of a profuse irritating discharge with itching and burning of the vagina and examination reveals an inflamed vaginal outlet with less severe diffuse inflammation of the whole vagina mucosa Of 29 patients who were given large doses of cod liver oil or halibut liver oil 16 were completely cured 9 showed marked improvement and 4 moderate improvement Most of the patients were cured or improved within 4–8 weeks and only one took as long as 20 weeks Of 13 patients who were given the usual topical treat ment without vitamin A only one was completely cured and one much improved after being under observation for at least 44 weeks Four others showed only slight improvement and the remaining 7 no improvement

Goitre at puberty Fasold [38] had occasion to record the menses of seven young German girls aged 13 15 years who lived in a goitre area of the Black Forest In view of the suspected interaction between vitamin A and thyroxine he proceeded with some success to treat their swollen necks by the administration of large doses of vitamin A concentrate In four of the girls menstruation was suppressed and did not recommence until the vitamin therapy was stopped 7 months later An interrelationship between the functions of the ovaries thyroid (Chap 37) and vitamin A was therefore suggested

Premenstrual tension Heavy vitamin A therapy has also been found in recent observations by the Brazilian workers Argonz and Abinzano [39] to be beneficial in the treatment of disorders classed as

References p 523

premenstrual tension Thirty patients, suffering from mastodynia, tenderness of the abdomen, oedema and various nervous or psychological disorders were treated with daily oral doses of 200,000 i u of vitamin A during the second half of the menstrual cycle The therapy was stopped at the onset of each menstruation, and the same procedure was followed for 2–6 months In most of the patients the mastodynia disappeared, mammary nodules disappeared or were decreased in size, oedema was corrected and nervous disorders were improved The cures continued after therapy had been stopped and the disorders did nor reappear within a year of the commencement of dosing No improvement was observed in patients who were given placebos or vitamin A irregularly or only for short periods

Summary of the Interrelationships between Sex and Vitamin A

The evidence which has been reviewed in the present and preceding chapters indicates that vitamin A affects the sex functions, and conversely that sex factors influence the metabolism of vitamin A The numerous scattered observations on which this evidence is based, which in combination appear to make an impressive case, may be grouped together and summarised as follows

(a) *Requirements for vitamin A and rates of expenditure of reserves* The incidence of night blindness has been found to be much higher in men than in women In rats kept on diets low in vitamin A mortality rates are higher in males than in females The liver reserves tend to be used up more rapidly in male rats than in females

(b) *Distribution of vitamin A in males and females* The liver reserves of vitamin A have been found to be higher in cows than in bulls Female rats tend to have higher concentrations than males in their livers, and lower in their kidneys and their blood In groups of normal men the average level of vitamin A in the blood plasma tends to be higher, and that for carotenoids lower than in women For vitamin A a similar sex difference may be seen in the maximum levels reached after the administration of massive doses

(c) *The effect of sex hormones* In castrated or immature rats given suitable doses of vitamin A injections of oestradiol have been shown to reduce the concentration of vitamin A in the kidneys from high levels, typical of males, to low levels, typical of females In immature pullets oestrogens have been found to raise the level of vitamin A in the blood, but this effect has not been observed in mammals

(d) *Effect of vitamin A deficiency on the sex organs* The epithelia of the testes and vagina are both highly vulnerable to the effects of vitamin A

deficiency In female rats vaginal cornification is the earliest sign of vitamin A deficiency To maintain the regularity of the oestrous cycle larger doses of vitamin A are necessary than for survival and growth

(e) *Antagonism between vitamin A and oestrogens* The presence of vitamin A in the diet is necessary for the production of mucus by the vaginal epithelium In the normal oestrus cycle this activity is interrupted by the periodical intervention of oestrogens which check the formation of mucus and cause the appearance of the cornified cells typical of either oestrus or vitamin A deficiency Evidence that this interrelationship may be to some degree specific has been found in the ability of daily massive doses of vitamin A to cause the persistence of mucus formation in ovariectomised rats even after an oestrogen had been injected Conversely the continuously cornified vaginal smears of rats injected with oestrogens have been changed with the appearence of mucus by single massive doses of vitamin A

Some clinical evidence has been obtained which suggests that heavy doses of vitamin A may be beneficial in the treatment of some types of vaginitis and menstrual disorder in women

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CHAPTER 37

Vitamin A and the Thyroid

Possible relationships between vitamin A and the thyroid gland have been studied so intensively that a chapter may well be devoted exclusively to this subject This recognition of an interesting field of research, however, must not be taken as implying that a specific interaction between vitamin A and thyroxine can be accepted as an established fact It seems probable, indeed, that many instances of presumed interaction can be explained by the thyroid gland influencing the basal metabolic rate, and the B M R in turn influencing the metabolism of vitamin A Reports that vitamin A may counteract an excess in metabolic rate, however, cannot be explained in this way

Broadly speaking the evidence for an interrelationship between the thyroid and vitamin A can be classified under three headings (1) Claims that in various ways vitamin A and thyroxine are antagonistic in their action (2) Claims that thyroxine is concerned in the conversion of carotene to vitamin A and (3) Claims that thyroxine influences the storage of vitamin A, and the rate at which the vitamin is used up Indications that thyroxine acts in one direction can usually be matched by evidence that inhibitors act in the reverse direction

The Alleged Antagonism between Vitamin A and Thyroxine

The thyroid in relation to vitamin A deficiency

An antagonism between thyroxine and carotene was suggested in 1932 by von Euler and Klussman [1], who found that the two substances had opposite actions on the growth of rats deficient in vitamin A. Thus dosing with carotene promoted growth, but thyroxine accelerated the decline of the animals The effect of thyroxine in inducing vitamin A deficiency was confirmed by observations by Sure and Buchanan [2] on the onset of xerophthalmia, and by Greaves and Schmidt [3] on vaginal keratinisation In the reverse direction thyroidectomy, or dosing with thyroid inhibitors, ameliorated the

References p 532

effects of vitamin A deficiency This effect was observed by Wiese, Mehl and Deuel [4] in experiments in which the lives of rats deprived of vitamin A were greatly prolonged by the administration of thiouracil Cooper, March and Biely [5] found that thiouracil could also improve the growth rate of chicks given small doses of vitamin A Recent findings by Blaizot and Benac [6] that the oxygen consumption of rats is increased in deficiency of vitamin A agree with the principle that thyroxine should aggravate the effects of avitaminosis, and that thyroxine antagonists should be beneficial

Thyroxine therapy in hypervitaminosis A According to Fasold and Peters [7] thyroxine was effective in rats in counteracting the effects of great excess of vitamin A (Chap 28) This finding agreed well with the action of the hormone in aggravating the effects of deficiency Baumann and Moore [8], however, failed to confirm that massive injections of thyroxine could counteract the effects of hypervitaminosis A In their experience rats suffered more from combined excess of vitamin A and thyroxine than from either excess alone Possibly thyroxine may be beneficial in vitamin A excess when it is given in moderate doses If the dosing is too drastic, however, any effect of thyroxine in facilitating the disposal of vitamin A will be outweighed by its own toxicity

Thyrotoxicosis treated by vitamin A Besides claiming that thyroxine was beneficial in hypervitaminosis A Fasold and Peters [7] also asserted that vitamin A, dissolved in arachis oil could prevent the toxic effect of excess of thyroxine A similar conclusion had been reached by Abelin Goldener and Kobori [9] and confirmatory evidence was later available from many sources [10–14] According to Wendt [15] vitamin A reduced the excessive metabolic rate, and ameliorated the other symptoms, in human patients suffering from hyperthyroidism Dietrich [16] and Fasold [17] agreed with these claims, but Catel [18] was unsuccessful in the treatment of a child with hyperthyroidism Logaras and Drummond [19] found that the increased metabolism induced by injections of 100 μg of thyroxine into rats receiving a basal diet deficient in vitamin A was partially prevented by liberal doses of 3000 i u of the vitamin but not by marginal doses of 10 i u Increased metabolism caused by dinitrophenol was not prevented by vitamin A Sheets and Struck [20] also reported that massive doses of vitamin A concentrates tended to reduce the metabolic rate in rats given thyroid In their experiment, however, the effect was only slight and not statistically significant

It must be noted moreover that Fasold and Peters [7] found that arachis oil used as a solvent for vitamin A, could protect against excess of thyroxine even when given by itself It seems questionable, therefore, to what degree the protective action of vitamin A is specific The possibility remains open

that the protection wholly or in part may sometimes be due to the medium in which the vitamin is administered

Similar doubts as to specificity must be raised in regard to the ability of the vitamin to interfere in the Reid Hunt test in which thyroxine is tested by its ability to protect rats from poisoning by acetonitrile According to Fleischmann and Kann [21] excess of vitamin A can reduce this protection Ether extracts of normal human blood serum have the same action but Hochstadt and Malkiel [22] found that vitamin A and the inhibitory factor could be separated into different fractions Thus when the serum was extracted for one hour the extract contained vitamin A but had little antithyroidal activity In extracts obtained by long extractions the vitamin was absent presumably having been oxidised but high antithyroidal activity was observed

Influence of vitamin A on the condition of the thyroid gland

Further evidence of an antagonism between vitamin A and thyroxine or at least of some form of interaction may also be seen in the influence of the vitamin A status on the size and histological condition of the thyroid gland Changes have been reported to occur both in deficiency of vitamin A and in excess [23 25] In deficiency Coplan and Sampson [26] found that the thyroid was hypertrophied in female rats but atrophied in males Toxic overdosage with the vitamin according to Carpenter and Sampson [27] also caused striking changes Thus the follicles which contained only small amounts of colloid were reduced in size and irregular in shape In contrast the follicular cells were hypertrophied The uptake of radioactive iodine by the abnormal glands appeared to be increased and was particularly intense in the smaller follicles Opposition to the action of the thyrotrophic hormone of the pituitary by large doses of vitamin A was reported by Schneider [28] and by Fellinger and Hochstadt [29]

Metamorphosis

In the development of salamander larvae (axolotls) vitamin A and carotene have been reported to counteract the normal effect of thyroxine in stimulating metamorphosis [21 30]

The Influence of Thyroxine on the Conversion of Carotene

Conversion in vivo

A dramatic indication that the thyroid is concerned in the conversion of carotene was reported in 1933 by Fasold and Heidemann [31] The milk fat of the goat is usually white like that of the sheep but after thyroidectomy it became yellow Unfortunately this observation has never been confirmed In the reverse direction Parhon

References p 532

and Werner [32] found that thyroxine lowered the level of carotenoids in the blood of drakes According to Wendt [33] carotene and also vitamin A were low in the blood of human patients with hyperthyroidism Conversely Josephs [34] observed high levels of carotenoids in the blood of children with hypothyroidism In the experience of Remington *et al* [35] but not in that of Drill and Truant [36] carotene was converted by thyroidectomised rats efficiently enough to allow small doses of the provitamin to cure their xerophthalmia

Systematic and quantitative studies with rats were made in 1947 by Johnson and Baumann [37] Weanlings were kept on a diet deficient in vitamin A for 4 weeks Some received desiccated thyroid tissue others thiourea or thiouracil while others served as controls Under the same conditions daily doses of carotene were given for a further 15 days and the animals were then killed for the estimation of vitamin A in their livers and kidneys The following results for the total vitamin in both organs were obtained with 40 μg doses of the provitamin

	B M R	*Total vitamin A* i u
Control		173
Thyroid	+77%	305
Thiourea	—26%	15
Thiouracil	—22%	26

In parallel experiments the thyroid inhibitors were found to have little effect on the storage of preformed vitamin A It seemed reasonable therefore to conclude that the conversion of carotene to vitamin A was disturbed The somewhat increased storage of vitamin A in the animals treated with thyroid suggested that extra thyroxine could induce an efficiency of conversion above the normal level

Attempts to confirm these findings have met with varying degrees of success Kelley and Day [38] obtained different results according to whether the carotene was given as a single dose or as repeated doses When a single dose was given the liver stores of vitamin A were somewhat larger in rats given thyroxine than in rats given thiouracil but after repeated doses of carotene the same stores were found for both treatments It was suggested that in the second type of experiment the reduced efficiency of conversion of carotene under the influence of thiouracil was counterbalanced by the known effect of this drug in retarding the depletion of reserves of vitamin A from the liver

In experiments by Morgan and White [39] thyroxine increased the size of rats livers by some 30% The hormone also caused an increase to the same

extent in the total vitamin A storage which resulted from repeated doses of carotene Later Arnrich and Morgan [40] obtained results which appeared to contradict those of Johnson and Baumann, since thiouracil considerably increased the storage of vitamin A in rats dosed with carotene Their rats were first dosed for seven weeks with 440 μg daily of carotene, with or without thiouracil, and were then kept on their basal diet, deficient in vitamin A, for another two weeks The total vitamin A reserves averaged about 900 i u in the thiouracil group and only 350 i u in the control group. Thiouracil had no effect on the vitamin A stores, however, when higher doses of carotene were given

Further evidence emphasised the need for caution in accepting a specific role for thyroxine in the conversion of carotene In pigs which had been dosed with carotene Swick, Grummer and Baumann [41] found slightly higher stores of vitamin A in animals which had been treated either with thyroprotein or thiouracil than in control animals This greater storage was presumably associated with the reduced growth rate which was caused by either treatment, and which decreased the expenditure of the vitamin Using aqueous suspensions of carotene Bieri and Schultze [42] were unable to detect significant differences in the storage of vitamin A by normal rats and by others treated with thiouracil The only divergence was found in a higher level of carotene in the blood of the normal rats after the carotene had been given by intramuscular injection Even this difference was not observed after the provitamin had been administered orally

Against these negative findings, however, further evidence for the importance of the thyroid in carotene conversion was reported from Belgium by Kowalewski, Henrotin and Van Geertruyden [43] Injections of carotene into the portal vein of thyroidectomised dogs increased the level of the pigment in the blood and liver, but there was no formation of vitamin A, as in normal dogs Thyroxine accelerated the conversion of carotene in normal dogs

The alleged conversion of carotene in vitro *by thyroxine*

Evidence which, if confirmed, would have amounted almost to conclusive proof of the direct intervention of the thyroid in the conversion of carotene was advanced in 1946 by Kaplansky and Balaba [44]. It was claimed that when carotene in colloidal solution was incubated with thyroglobulin or iodinated casein it was converted, in good yield, to vitamin A In numerous attempts, however, Cama and Goodwin [45] failed to confirm the conversion by iodinated casein They could only conclude that the Russian workers had been mislead by the development of the cis peak at 326 mμ as the result of isomerism during the incubation of their carotene Equally negative results were obtained by McGillivray [46] and Lowry and Lowry [47].

References p 532

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References p 532

The efficiency of absorption of carotene

In attempting to explain the various reports of the influence of the thyroid on carotene metabolism Cama and Goodwin [48] considered three possibilities Thus thyroxine might affect (1) the enzyme system in the intestines responsible for the conversion of carotene (2) the stability of carotene in the intestines or (3) the absorption of carotene from the intestinal lumen Experiments with rabbits failed to demonstrate any effect of either thyroxine or thiouracil in influencing the level of vitamin A in the blood and there was no evidence of the passage of either carotene or retinene into the blood stream The first possibility therefore seemed unlikely No stabilising effect on carotene could be demonstrated for thiouracil *in vitro* which seemed against the second possibility In favour of the third alternative however the results of a limited number of experiments with rats suggested that thyroxine increased and thiouracil decreased the efficiency of absorption Thus in rats given desiccated thyroid the faecal excretion of carotene was decreased on an average to the extent of 18% below the excretion in control animals The administration of thiouracil increased the excretion by an average of 31%

Further evidence that the thyroid may influence the absorption of carotene was reported by Chanda *et al*[49] Cows in which the faecal excretion of carotene had been reduced by restriction to a deficient diet were dosed with dried grass meal and in some cases were also injected with thyroxine or thiouracil The appearance of carotene in the faeces was delayed by thyroxine and accelerated by thiouracil The apparent digestibility of the carotene in agreement with these observations was somewhat increased by thyroxine and decreased by thiouracil Similar results were obtained with goats In later experiments Chanda and Owen [50] found that thyroxine increased and thiouracil decreased the carotene and vitamin A contents of cows milk which supports the theory of differences in the rates of intestinal absorption

The Influence of Thyroxine on the Storage and Expenditure of Preformed Vitamin A

Storage

Less evidence is available on the effect of the thyroid status on the storage of vitamin A than on the conversion of carotene In most experiments little difference has been found between the storage in normal animals and others in the *hypo* or *hyper*thyroid state There are some indications however that excess of thyroxine may tend to increase the storage of the vitamin

In rats given daily doses of 3000 i u of vitamin A Logaras and Drummond [19] found that treatment with either thyroxine or dinitrophenol in

creased the average storage of the vitamin by 15–20% over the level found in untreated animals Johnson and Baumann[37] who gave 130 i u daily found little difference in storage by normal *hyper* and *hypo*thyroidal rats Morgan and White[39] found that the stores accumulated by rats given about 100 i u of the vitamin daily for six weeks were the same in controls and in animals given thyroid The equality in the total stores was maintained even although the livers were enlarged by the treatment with thyroid

Indications that thyroxine may increase the storage of vitamin A whether derived from the preformed vitamin or from carotene may be seen in the high reserves which have often been found in patients who have died from exophthalmic goitre In limited numbers of cases both Wolff[51] and Moore[52] found that the reserves were usually above the normal average

The expenditure of vitamin A Careful experiments on rats by Johnson and Baumann[37] indicated that thyroxine increases the expenditure of the vitamin A reserves during restriction to a deficient diet The interpretation of results however was complicated by the influence of growth in increasing the expenditure of the vitamin Thus in both normal and thyroid treated rats the expenditure of vitamin could be reduced by restricting the food intake which in turn restricted growth It was concluded that a three fold increase in the metabolic rate could be more than counter balanced by a decrease in the growth rate by 50%

The importance of growth as a complicating factor was emphasised by Heimer Maslow and Sobel[53] Rats which had been allowed to accumulate stores of vitamin A were thyroidectomised treated with thyroid or left as controls After a period of restriction to a deficient diet more vitamin A remained in the livers of either the *hypo* or *hyper*thyroid animals than in those of the controls In agreement with the effects in decreasing the ex penditure of vitamin A growth was much slower in both the treated groups than in the control group In this investigation no account was taken of the possibility recognised by Johnson and Baumann that growth may cause a migration of vitamin from the liver to the kidneys

Conclusions

A review of all the evidence which has been outlined in the preceding sections can leave little doubt of the ability of the thyroid to influence the metabolism of vitamin A and of provitamins The degree of specificity of this influence however remains undefined It is well known of course that the blood lipids may be reduced in hyperthyroidism and greatly increased in hypo thyroidism With such pronounced changes in some of the major consti tuents of the body it is perhaps not surprising that minor constituents such

Refere ces p 532

as vitamin A should drift with the metabolic tide As a working hypothesis it seems reasonable to assume that the usual effect of thyroxine will be to accelerate any prevailing trend in vitamin A metabolism Thus if dietary conditions are conducive to the storage of vitamin A the amounts stored will usually be increased by thyroxine If conditions are conducive to expenditure then the rate of expenditure will be increased Thyroid inhibitors will usually have the reverse effects We have seen however that in some types of experiment the influence of the thyroid on vitamin A metabolism may overlap the influence of growth

Evidence that vitamin A can oppose the action of thyroxine in its effect on the metabolic rate might appear to advance a stronger case for a specific antagonism But some disagreement remains about whether the vitamin regularly opposes the action of thyroxine and if so whether other lipoids can have the same effect Progress appears to wait on an increase in our knowledge of the mode of action of thyroxine Possibly the theories of Martius [54] on the influence of the hormone on oxidative phosphorylation will allow a start to be made in this direction

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CHAPTER 38 ✓

Vitamin A in Relation to Various Biochemical or Nutritional Mechanisms, including Detoxications

The greatest progress in elucidating the biochemistry of vitamin A has been achieved in studies on its role in the retina (Chap 22) In certain Crustacea most of the vitamin A contents of the body are concentrated in the eyes [1], and it might reasonably be inferred that the main role of vitamin A in these animals is concerned with the visual processes In most vertebrate animals, however, the amount of vitamin A present in the retina makes up only a minute fraction of the total vitamin A contents of the organism No evidence has been advanced, moreover that vitamin A is oxidised to its aldehyde, a key substance in vision, elsewhere than in the retina The importance of the vitamin in the general system, therefore, must be explained by a biochemical mechanism which differs from that which has been studied, so successfully, in the eye

Forty years after the recognition of the existence of vitamin A there still seems no clue as to its general point of attack in a biochemical sense, although there is adequate anatomical and histological knowledge (Chaps 24–28) about the sites which are affected by deficiency. At the most we can say that certain systems, such as those controlling the formation of mucus and the detoxication of poisons, can be affected by the vitamin A status In many instances we cannot even be sure that the role of the vitamin is specific, or that the effects under investigation are not due merely to a general improvement in health With these reservations however, it may be helpful to collect together such information as is available

Vitamin A and Mucus Formation

The formation of mucus In experimental deficiency of vitamin A, as seen in the rat and other animals the drying up of mucous membranes is a prominent feature As explained in Chapter 36 the mucus-producing effect of vitamin A may conveniently be studied in the vaginal epithelium, where it appears to be opposed periodically by the

intervention of oestrogens which cause the formation of keratinised scales Deficiency of the vitamin however, also affects other mucous membranes throughout the body

Composition of mucus Consideration of the chemical nature of mucus, therefore may well provide a clue to the biochemical action of the vitamin in one of its most important functions According to Meyer [2] the mucoids and glycoproteins vary greatly in their contents according to their source When they contain more than 4 per cent of an amino derivative of a sugar such as acetyl glucosamine, they are classified as mucoids If the level of sugar derivative is lower, however they are classified as glycoproteins In addition to glucosamine members of both groups contain polysaccharides glucuronic acid and other organic acids sulphuric acid and protein in various selections and combinations If we consider the role of vitamin A on the widest possible basis, therefore, we may say that it is neces[illegible] glucosamine In its abse[illegible] the unbalanced production of keratin, a protein rich in sulphur which differs from the mucoids and glycoproteins in being insoluble in water

Cartilage and bone Apart from the mucous membranes some further hints may perhaps be gained from a study of the effect of excess of vitamin A on bone formation Wolbach and Maddock [3] have described the first effect of hypervitaminosis A on the bones of young rats as an increased rate of maturation and vascular penetration of the epiphyseal cartilage cells Normal cartilage consists of the insoluble protein collagen in association with the chondroitinsulphuric acid which is a polysaccharidic acid containing equal amounts of glucuronic acid and acetylchondrosamine sulphuric acid [4] If it could be proved that vitamin A influences the relative rates of formation of these two components we would have another example of its ability as found in mucous membranes to maintain a balance between soluble mucoids and insoluble scleroproteins

Intestines An indirect investigation of the importance of vitamin A in mucin metabolism was made in 1937 by Manville [5] Since the mucosa of the gastro intestinal tract was considered to be more exposed to injury than any other form of mucous epithelium it seemed probable that it might be particularly vulnerable to deficiency of vitamin A The mucus-secreting goblet cells were therefore counted in comparable areas of entire [illegible]

References p 542

Vitamin A and Detoxication

Menthol poisoning To obtain further evidence for validity of this theory Manville examined the pathological effects of interference in mucus formation by dosing rabbits with menthol This substance is detoxicated by combination with glucuronic acid, a constituent of mucin which may be obtained either endogenously from glycogenic amino acids or exogenously from the diet By the frequent administration of menthol by stomach tube to the animals, while they were kept on a diet of oatmeal and water, the capacity of the detoxication mechanism was exceeded presumably because the combination of endogenous and dietary supplies of glucoronic acid could not keep pace with the greatly increased demand In those rabbits which survived the treatment for a few days ulcers and erosions were found in the stomach, pylorus, gall bladder and small and large intestines These injuries were considered to resemble closely the corresponding lesions caused by deficiency of vitamin A

Detoxication of sodium benzoate In the above experiments the doses of menthol were sufficiently heavy to produce conditions resembling vitamin A deficiency *in* animals *which* presumably still possessed the usual reserves of the vitamin in their livers In more recent work by Meunier and Ferrando [6] less massive doses of a toxic substance in this case sodium benzoate, were given to rats receiving different doses of vitamin A Table 58 shows the results obtained in rearranged form

TABLE 58

INFLUENCE OF SODIUM BENZOATE ON THE REQUIREMENTS OF RATS FOR VITAMIN A (MEUNIER *et al* 1949)

Group No	*No and sex of rats*	*Benzoate in diet (2%)*	*Glycine 150 mg/rat daily*	*Daily dose of vitamin A i u*	*Mean period of survival (days)*	*Mean weekly weight gains (g)*
2	4 M	—	—	0	78	1 87
1	3 M	—	—	8	∞	4 60
8	4 M	—	—	66	∞	8 26
3	3 M	+	—	0	20	0 16
5	4 M	+	—	8–50	55	1 73
7	3 M	+	—	33	21	1 15
10	4 F	+	—	66	∞	5 70
4	5 M	+	+	0	101	2 38
6	3 M	+	+	8	∞	3 24

It will be seen that the animals declined and died when given benzoate in conjunction with doses of vitamin A which would otherwise have just sufficed for moderate growth, but grew rapidly when given the benzoate in

conjunction with an increased allowance of vitamin A Alternatively the animals could be made to grow by giving glycine in amounts sufficient to detoxicate the benzoate One possible criticism of these interesting experiments is the use of female rats in the crucial group 10 since it cannot be assumed that the sexes are necessarily equally vulnerable to poisoning by sodium benzoate

Ferrando [7] extended these investigations in a study of the effect of vitamin A deficiency on the urinary components of rats given sodium benzoate The mean increases in the amounts of nitrogen in its various forms and of glucuronic acid which were induced by the administration of benzoate were as follows

	N in mg per 24 hr					
	Total	*Hippuric acid*	*Urea*	*Creatinine*	*Creatine*	*Glucuronic acid mg/24 hr*
+Vitamin A	3 7	3 84	6 02	0 11	—0 02	27 1
—Vitamin A	24 3	2 39	24 24	0 24	0 06	4 97

Thus the animals given vitamin A excreted the greater amounts of hippuric acid and glucuronic acid but less total nitrogen urea creatine and creatinine than the deficient animals Ferrando regards the conjugation of benzoate with glucuronic acid as a sortie de secours additional to its detoxication as hippuric acid by combination with glycine and it would appear that this mechanism is severely affected by the absence of vitamin A The larger increases in creatine and creatinine in the deficient animals suggest that the benzoate causes destruction of the muscles when not counterbalanced by an increased allowance of vitamin A The excess of the sum of the sum of the nitrogen components over total nitrogen particularly in the groups receiving vitamin A seems however to indicate considerable analytical difficulties

Detoxication of monobromobenzene Haley and Samuelsen [8] reported that rats which had been depleted in vitamin A invariably died within 48 hours after an injection of 100 mg of monobromobenzene dissolved in maize oil Normally nourished animals showed no harmful effects It was also found that when the bromobenzene was mixed with the diet for some weeks the storage of vitamin A in the liver as measured by the method of Rosenthal and Erdélyi (Chap 7) was less than in animals not given the bromobenzene This effect was not produced however, when the bromobenzene was injected The authors concluded from the second experiment that vitamin A was not directly associated with the detoxication

References p 542

of bromobenzene, but that this substance interfered with the absorption of the vitamin

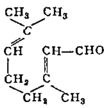
Later Meunier and his colleagues [9] carried out experiments with bromo benzene in parallel with those which they had already made with sodium benzoate The rats used were all males, and it was again found that the effect of the poisoning could be overcome by a large dose of vitamin A The protective action of small doses against the poison was not examined Since bromobenzene is detoxicated mainly by combination with cystine trials were also made of the detoxicating powers of supplements of this amino acid In contrast to the efficacy of glycine in protecting against benzoate poisoning, however, it was found to be ineffective The results obtained are given in Table 59

TABLE 59

INFLUENCE OF BROMOBENZENE ON THE VITAMIN A REQUIREMENTS OF RATS (MEUNIER *et al* 1950)

Group No	*No and sex of rats*	*Bromo-benzene in diet (1%)*	*Cystine in diet 120 mg%*	*Daily dose of vitamin A i u*	*Mean period of survival (days)*	*Mean weekly weight gain (g)*
1	5 M	—	—	0	34	+0 88
2	5 M	—	—	8	∞	+4 89
3	4 M	+	—	0	25	—1 14
4	5 M	+	—	66	∞	+6 94
5	5 M	+	+	0	25	—0 86

Citral poisoning At this point it may be appropriate to mention recent work by Leach and Lloyd [10] on the possible relationship between citral poisoning and vitamin A deficiency The story differs from those concerning the detoxication of benzoate or of bromobenzene, however, in that the doses of citral claimed to cause toxic effects were very much smaller The possibility has been suggested that citral may owe its toxicity to the similarity of its structure to part of that of the vitamin A molecule, which causes it to act as an antivitamin



Monkeys were used as experimental animals This species was reported to develop eye lesions in vitamin A deficiency by Hetler [11], although in the experience of Tilden and Miller [12] the animals can die of colitis without

developing xerophthalmia. According to Ramingalaswami, Leach and Sriramachari [13] severe ocular lesions are sustained, which include keratomalacia and degenerative changes in the rods and cones, pigment epithelium and Descemet's endothelium.

In the course of studies of poisoning by substances other than citral, which produced lesions resembling those seen in vitamin A deficiency, Leach and Lloyd [10] had reason to suspect that all was not well with their control animals. The eyes of monkeys which had received a synthetic diet appeared to be normal, but in those of a control animal, which had been given oranges, changes suggestive of vitamin A deficiency were observed. It was surmised that the injurious substance in oranges was citral, and tests of its toxicity were therefore carried out Even daily doses of 1 μg per kg of body weight caused damage to the vascular endothelium. In rabbits single injections of 5 μg per kg were harmful. A typical effect of the citral was to cause damage to the trabeculum and canal of Schlemm, which resulted in raised intra-ocular pressure

The poisonous action of citral could be prevented or reversed by large doses of vitamin A Other aldehydes, including cinnamic aldehyde and crocetin aldehyde, were also toxic. Protection was given by sulphydryl substances and by aldehyde trapping agents.

The further development and clarification of this work will be awaited with interest

X-disease in cattle From Chapter 34 it will be remembered that Ferrando has suggested that this disease may be considered as a parallel in the field to the experimental poisoning of rats by sodium benzoate or bromobenzene Thus in the affected bovines the vitamin A status is disturbed by chlorinated naphthalenes, or other poisons, concurrently with the development of the symptoms of the disease In the rat a moderate vitamin A intake which would normally suffice for growth, is made inadequate by the presence of the poisons

The conception that the vitamin A requirement may be influenced by metabolic stress raises important issues If extraneous poisons [illegible]
the vitami [illegible]
may have [illegible]
further research [illegible]

Miscellaneous Effects of Vitamin A

Vitamin A in protein deficiency From their evidence of the value of a liberal allowance of vitamin A for the efficient working of detoxication mechanisms the French workers inferred that it plays

References p 542.

an important role in protein metabolism Bearing this suggestion in mind Moore Sharman and Ward [15] have made a preliminary trial of the value of vitamin A in enabling rats to subsist on a diet grossly deficient in protein

Male rats were first kept upon a diet adequate in protein but deficient in vitamin A which was supplemented after the first few weeks with small doses of the vitamin The allowance of about 4 i u daily permitted growth to weights of over 200 g in about 3 months but was known to be inadequate to allow the accumulation of reserves in the liver The animals were then divided into two groups one of which continued to receive only 4 i u daily while the other was given 15 000 i u of the vitamin during 3 days followed by weekly doses of 1000 i u Both groups were transferred to a diet in which the only source of protein was 10 per cent of dried yeast Any danger of deficiency of vitamin E which is well known to affect the resistance of the rat to protein deficiency [16 23], had been prevented by giving adequate doses of tocopherol from the commencement of the experiment The weight changes during the next 10 weeks were as follows

Dosage of vitamin A	*Average wt change g*
Low	—30 g (for 3 survivors)
High	+ 9 g

The animals kept on a low allowance of vitamin A steadily lost weight and one of them whose weight has not been included in the average eventually died In contrast the rats given liberal doses increased temporarily in weight and remained for at least the next 3 months slightly heavier than at the commencement of protein deficiency In order to prove that the inability of the rats on the lower dose of vitamin A to maintain their growth was not due solely to a subnormal intake of vitamin A continued over such a long period an adequate allowance of casein was restored to their diet after 10 weeks of protein deficiency Without any alteration in the vitamin A allowance all the animals immediately commenced to put on weight rapidly

It would be premature of course to claim from these results that vitamin A plays a specific role in protein metabolism Possibly the old axiom that growth is limited by the single factor which is least adequately supplied in the diet is not strictly true Rats may thrive less well when they are suffering from two forms of partial deficiency even unrelated than when they are suffering from a single deficiency

Vitamin A and restricted food intake

Patterson McHenry and Crandall [24] compared the body weights of rats which had been kept for 6 weeks on a diet deficient in vitamin A with those of other rats given the same amount of food in conjuction with adequate doses of

the vitamin The weights of the animals which had been given the vitamin were 15 to 25 per cent greater than those of the animals without supplements. Analyses of the bodies indicated that 52 per cent of the difference was due to fat, 16 per cent to protein and 32 per cent to water. Under the conditions of these experiments, therefore, the efficiency of the food in producing or maintaining the tissues was increased by the presence of vitamin A.

Later Mayer and Krehl [25] reached the same conclusion from experiments which were not based on the "paired feeding" procedure Even in rats which were dosed with the vitamin the quotient of "weight gain" divided by "food intake" decreased sharply as the animals became larger, as is inevitable on account of the termination of growth at maturity The effect of vitamin A deficiency, which became obvious much sooner in males than in females (Chap 35) was seen in a reduction in the efficiency ratio below that found in animals dosed with vitamin A which had been started upon the experiment at the same time With the premature cessation of growth in the deficient animals the ratio fell to zero.

Evidence suggesting that some components of the diet make heavier demands than others on vitamin A metabolism was put forward by Selye [26]. When he restricted guinea pigs to a diet consisting mainly of carbohydrates they died within about 30 days without developing xerophthalmia or pronounced scurvy A diet of hay shortened the survival period to 18–23 days, with the appearance of xerophthalmia in all the animals and with more or less plain signs of scurvy. An unbalanced diet consisting entirely of vitamin-free casein, however, had a much more drastic effect, causing severe xerophthalmia in 5–6 days and death in 6–7 days Presumably the animals died before scurvy had time to develop Possibly the only safe conclusion to be drawn from these experiments is that the liability of the eyes to conditions which resemble xerophthalmia at least superficially, may be influenced by factors other than vitamin A

Cholesterol metabolism The possible influence of vitamin A on cholesterol metabolism was studied by Lasch [27] who noticed that the sterol lev[illegible]

from various dis[illegible]

had been given [illegible]

mality in the levels of cholesterol in the blood plasma and livers of rats deficient in vitamin A

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CHAPTER 39 ✓

Assessment of our Present Knowledge of Vitamin A

As our book nears its end the author must repeat from the Preface his apologies to those workers whose investigations he has overlooked or has reviewed only inadequately Research has been so prolific that several volumes and several authors would be necessary in order to cover all its aspects comprehensively For the same reason there will be no intention, in this concluding chapter to provide a full summary of all the information which has already been given No more will be attempted than to emphasise or amplify some of the salient features of our present knowledge of the vitamin and at the same time to indicate some of the gaps where information is lacking The opportunity will be taken to mention briefly any interesting developments which have occurred since the bulk of the book was written

Chemistry and Estimation

The completeness of our knowledge of the chemistry of the orthodox all *trans* vitamin A_1 is exemplified by the ability of manufacturing chemists to produce the pure substance in enormous quantities and at competitive prices We have equally good information on many of its derivatives some of which retain the physiological activity of the vitamin while others do not In particular we are familiar with the chemistry of the important aldehyde retinene (or retinal) which may be considered a key substance in the mechanism of vision The organic chemist it would appear for the present has stepped beyond the realm of Nature in producing vitamin A acid which possesses growth promoting power without being convertible into the vitamin Research to decide whether dosing with the acid can cure night blindness as well as promote growth would be interesting Any intervention of the acid in the visual processes seems unlikely but for that matter the ability to promote growth could hardly have been expected

Isomerism The discovery of the *cis trans* isomers of vitamin A which are biologically important in regard to vision opened up new fields for the exploitation of chemical skill after the main object of the isolation

References p 559

and synthesis of the all *trans* form had been successfully achieved According to recent findings by Plack[1] the *cis-trans* isomers of vitamin A may be divided as follows into two groups by the speed of their reaction with maleic anhydride

Fast reacting group All *trans*, 9-*cis* and possibly 7-*cis**.

Slow reacting group 11-*cis*, 13 *cis*, 9–13 di *cis*, and 11–13 di *cis**

Vitamin A_2 has provided further exercise for the chemist, with a duplication in the absence of two hydrogen atoms of all the derivatives that are possible for vitamin A_1 The multiplicity of the substances that could be obtained by permutations of all the various minor variations has been emphasised in Chapter 11

Oxidation products Information is still scanty on the products, other than retinene, which are produced by the mild oxidation of vitamin A We may recall, again from Chapter 11, that the exposure to air of a solution of vitamin A causes the production of a succession of absorption bands, with maxima at about 275, 285, 297 and 311 mμ, which may be contrasted with 368 mμ for retinene In seeking substances which might be responsible for absorption in these positions we have only knowledge of the oxidation product hepaxanthin, absorbing at 275 mμ, which is believed to be the epoxide of vitamin A There certainly seems scope for studies of the products formed when the oxidation of vitamin A commences in the centre of the molecule, rather than at the end of the side chain

Provitamins The chemistry of the better known provitamins, including α, β and γ carotenes and cryptoxanthin, can be considered like that of vitamin A, as familiar ground Our knowledge is still incomplete however, in regard to lesser known provitamins, such as aphanin, echinenone and torularhodin The formation of epoxides, and of related oxidation products, has been studied more intensively in regard to carotenoids than in regard to vitamin A Most of the products, however, are more interesting to botanists than to workers in the vitamin field

Methods of estimation Turning to the estimation of vitamin A by ultra violet spectroscopy we must commend the efforts of those experts who have worked for so many years to devise a perfect routine method It seems that as one mountain peak of achievement has been reached, another has appeared to present a fresh challenge Thus methods have been worked out to allow correction for the absorption of substances which are unrelated to the vitamin, but problems still remain in dealing with mixtures of *cis trans* isomers and with mixtures of vitamins A_1 and A_2 In regard to the antimony trichloride reaction Bruggemann Krauss and Tiews[2] have suggested the production of blue colour is actually

* Karrer s system of numbering

due to the pentachloride which is present in the reagent as impurity Further information on this point will be awaited with interest but in the meantime it may be noted that antimony pentachloride was investigated by Kahlenberg[a] in studies on the production of colour by sterols as long ago as 1922

Research on the estimation of provitamins has also continued actively and has been one of the main interests of the author s colleague Dr V H Booth (Chapter 6 and Appendix)

STORAGE AND DISTRIBUTION

The role of the liver The storage of vitamin A in the liver is such a familiar and well established phenomenon that at times we may be inclined to forget some of its remarkable features It is difficult to think of any other nutrient for which such very adequate provision can be made for future needs Thus most humans in Britain accumulate enough vitamin A to last them for two years while heavily dosed rats can store about twenty times the amount which seems necessary to suffice for their natural span of life There seems to be no obvious clue moreover to suggest why the liver under normal conditions of nutrition should attract concentrations of vitamin which are so much in excess of those in other organs

Vitamin A is soluble in fat and we might expect a fairly even distribution throughout the fat of the body Subject to minor qualifications such an even distribution seems to hold good for vitamin E which resembles vitamin A in being a fat soluble alcohol We have seen that the Kupffer cells play a large part in the storage of vitamin A If the reticulo endothelial system holds the key of the predominance of the liver in vitamin A storage however why do we find only traces of vitamin in the spleen and bone marrow? We may mention again the point from Chapter 28 that if the high concentrations of vitamin A normally found in the liver could be suddenly shifted to some other site they would almost certainly be highly toxic In addition to its high storage capacity for vitamin A, therefore the liver seems to be resistant to hypervitaminosis

Mobilisation The nature of the mechanism by which vitamin A is mobilised from the liver into the blood plasma also requires further investigation although its effects seem reasonably clear Thus as one side of the picture we can visualise the action of the liver after a massive dose of vitamin A when it removes the excess of vitamin from the blood stream and stores it both in the main liver cells and in the Kupffer cells The newly absorbed vitamin appears to be esterified on adsorption from the intestines even if it has been ingested as the free alcohol and is stored in the liver predominantly in esterified form As the reverse process it seems reasonable

References p 559

to assume that the liver can release vitamin A back into the blood stream and so allow a constant level to be maintained even during dietary deficiency The mobilised vitamin is mainly, and possibly entirely, in the form of the free alcohol, and appears to be loosely attached to protein

We have seen that the amount of vitamin A carried in the blood is influenced remarkably little by increasing the liver reserve When the reserve is reduced, however, a point is eventually reached when the customary level in the blood can no longer be maintained Most workers agree that human subjects tend to maintain their own individual levels of vitamin A over long periods, although there are wide differences between individuals We have seen that sex undoubtedly influences the blood level of vitamin A with the average values for men slightly higher than for women There have been claims that the level of vitamin A in the blood may be raised throug

alcohol

artificially or incurred as the result of disease In rats, under suitably chosen experimental conditions, sex may become the deciding factor in determining whether vitamin A shall be stored mainly in the liver (females) or in the kidneys (males)

Transfer during reproduction In studying the distribution of vitamin A between mother and offspring we have noticed that the amounts of vitamin passed to the foetus are usually small, although there seem to be exceptions to this general rule The colostrum is a rich source of the vitamin, and substantial contributions to the offspring are also made in the milk The strain of lactation on the vitamin A status of the mother may be severe

Differences between species *When we turn to the distribution of vitamin A* in relation to natural history we cannot fail to be struck by the remarkable absence of preformed vitamin A from the vegetable kingdom It

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kingdom it appeared ...

confined to vertebrates but this theory has become obsolete Thus vitamin A occurs in substantial amounts in the octopus lobster and shrimps Recently Kon and his colleagues [4] have detected traces of preformed vitamin A in even lower forms of marine life, including the humble limpet *Patella vulgata* and other low molluscs Goodwin and Taha [5], however, made no mention of having detected vitamin A during careful studies of the carotenoid contents of limpets

According to Plack Fisher, Henry and Kon [6] those *cis* isomers of vitamin A which react slowly with maleic anhydride are predominant in marine

Crustacea Continuing their marine studies Kon and his colleagues[7] have reported the presence of an unusual form of vitamin A activity in herring eggs. In confirmation of Junker[8] the eggs were found by biological tests to contain 7–46 1 u. per g, but only about 2 1 u. per g could be detected by the antimony trichloride method After feeding rats upon herring eggs, moreover, the reserves which were accumulated in the livers agreed with the results of the biological, rather than of the chemical assays on the eggs To the list of alleged "hidden" forms of vitamin A (Chap 23, Table 36), therefore, we must now add a "herring egg factor" which does not react in the antimony trichloride reaction itself, but which gives rise to vitamin A after ingestion by the rat The possibility that it may resemble the "copepod factor" of Lane[9] seems worth investigation

BIOCHEMISTRY

Without claiming mathematical exactitude we may account for about 0 1% of the total vitamin A contents of the human body in the retina, and for perhaps up to about 5% in the blood, kidneys, adrenals, fat deposits and other non-hepatic tissues. The remaining 94 9% of vitamin is presumably immobilised in the liver

The retinal system It seems ironical that our knowledge of the biochemistry of the vitamin is adequate only in regard to the small amounts which are located in the eye The gaining of this knowledge has been made possible, mainly through the skill and acumen of Wald and Morton by the fortunate circumstance that the retina contains two derivatives of the vitamin which are readily detected by spectroscopic methods. Thus both retinene and rhodopsin can easily be recognised in small concentrations by delicate, but reliable methods. In Chapter 23 we have emphasised the interesting point that elsewhere than in the eye retinene is rapidly reduced to vitamin A The protein, opsin, must be presumed to act as an aldehyde trapping agent, which forces the equilibrium between vitamin A and its aldehyde against the normal bias towards virtually complete reduction Moreover, we must conclude, in view of the destruction of rhodopsin by light, that opsin acts as an aldehyde trapping agent only in the dark

The general system. Turning to the biochemistry of vitamin A in tissues other than the eye we may feel disappointed that less progress seems to have been made It must be remembered, however, that the relationship between carotene and vitamin A was established in this field. Undoubtedly suspicions of a connection between visual purple and the carotenoids had been roused even before the existence of vitamin A had been recognised, but the exact nature of the relationship between carotene and vitamin A could hardly have been established by studies on

References p 559

the retina alone. It is remarkable that nearly 30 years after the discovery of the conversion of carotene there is still room for diversity of opinion as to the mechanism of the change. Presumably the main difficulty has arisen from complete failure to devise a model biochemical system which will convert carotene *in vitro*. Even in the intact animal, we may remember, the system for converting carotene, although prompt in commencing its action, is limited in regard to the amount of carotene which can be converted in a given time. Discussion still continues on the question whether the carotene molecule is neatly cut in the middle, or is nibbled away from one end.

Perhaps the strongest bar to progress in studying the biochemistry of vitamin A, outside the retina, has been the absence of any easily recognised products of its metabolism, in parallel with retinene and rhodopsin. We know that if retinene is administered to rats it is converted to vitamin A before it is passed into the blood stream. Vitamin A acid, on the other hand, is neither converted to vitamin A nor absorbed unchanged, at least as far as can be judged by preliminary research (Chap. 23).

An unconfirmed hint of the natural occurrence of an oxidation product of vitamin A in the tissues was given some 15 years ago by Le Page and Pett [10], who observed absorption at above 275 mμ in extracts of the blood plasma of humans who had been heavily dosed with the vitamin. Provided that the formation of artifacts was avoided during chemical manipulation this observation seems to provide a valuable clue, which has never been adequately exploited. Preliminary studies by Moore and Ward [11] were made upon the lungs of rats which had been given massive doses of vitamin A. In lung extracts from rats which were killed 23 hours after dosing the maximum due to vitamin A at 325 mμ was observed, but there was also a slightly higher peak at 312 mμ. Inflections had appeared at 282 and 296 mμ. In rats killed after longer intervals the intensity of absorption fell at all wave lengths, but at one point, 160 hours after dosing, the absorption at 282 mμ exceeded that at 325 mμ. Again the possibility of the formation of artifacts cannot be dismissed. The lungs were chosen for study as a site which might be favourable for the oxidation of vitamin A, and the rats were deprived of vitamin E with the idea of increasing the sensitivity of the vitamin to oxygen.

PATHOLOGY

Primary and secondary lesions. As ... in Chapter 24 ... which can be s... deficiency of ... ver a very wide ... include dama... al tissues, bones and nerves. For ... seen that ... affected in many different ways, ... may range

up to complete blindness In the early stages of deficiency we may star with a biochemical lesion, in which vitamin A is not available for the syn thesis of rhodopsin in the retina At this point the abnormality shoul respond readily to therapy Dryness of the cornea and conjunctiva ma next ensue, and may lead to ulceration, infection, and permanent loss o sight through scarring of the cornea or through extrusion of the lens A an alternative fate, which is particularly common in cattle, the eye ma remain free from xerophthalmia, but may lose its sight suddenly by pinchin or twisting of the optic nerve The nerve injury, in turn, may be related t abnormalities in bone formation, which affect the relative sizes of th different parts of the skull

We have seen that the drying of membranes in various parts of th body may lead to secondary infections, which cannot necessarily be checke by restoration of the vitamin In the experimental rat the initiation o urolithiasis is a common sequel of vitamin A deficiency, and the continuatio of stone formation may lead to the death of the animal long after th dietary defect has been corrected The blindness in bovines, which has bee mentioned in the preceding paragraph, may likewise make its appearanc months after the diet has ceased to be deficient (Chap 26)

Reproduction Deficiency of vitamin A has also adverse effects on repro duction Complete deficiency will cause complete failure t produce young either through degeneration of the testes, through failur of the female to conceive or through the premature termination of pregnan cy Partial deficiency may cause congenital abnormalities, including absenc of the eyes extrusion of the brain, cleft palate, and malformations of th aortic arch (Chap 27)

Hypervitaminosis In addition to deficiency we have seen that great exces of vitamin is also highly injurious (Chap 28) Agai abnormalities in bone formation are an essential feature In the rat there is also a tendency to haemorrhage, which has suggested a similarity of the condition to scurvy The concensus of opinion is against a secondary deficiency of vitamin C but the animals have hypoprothrombinaemia which responds to treatment with vitamin K

Hypervitaminosis A resembles avitaminosis A in interfering with repro duction, and the ranges of congenital abnormalities which can be incurred seem to be much the same There is room for strong suspicion, however, that at least some of the congenital abnormalities caused by either deficiency or excess of vitamin A are not highly specific Thus similar injuries may be caused by lack of other nutrients, or by treatment with X rays The charac ter of the injuries may depend to a great extent on the stage of gestation which is affected, rather than on the nature of the toxic agent

References p 559

Hydrocephalus It has long been known, of course, that deficiency of vitamin A causes a rise in the cerebro spinal fluid pressure The observation of hydrocephalus in vitamin A deficient rabbits and also in two deficient infants, therefore fits well into the general pathological picture (Chap 26) Perhaps we need not be too surprised that toxic excess of the vitamin, administered to infants, has much the same effect (Chap 33) It seems probable that the production of fluid by the choroid plexus becomes excessive whenever the vitamin A content of the blood plasma deviates outside its normal range

The Mode of Action of Vitamin A

The rhodopsin system The biochemical action of several of the B vitamins can be explained by their activity as components of enzyme systems In the retina vitamin A may also be linked up with an enzyme system, but as the substrate rather than a part of the enzyme itself Thus in Chapter 22 we have seen that the reduction of retinene to vitamin A may be catalysed by an enzyme which is present in the rod outer limbs and which requires cozymase, a derivative of the vitamin nicotinamide as its activator The rhodopsin system seems designed to function as a natural photo-cell, which can adjust itself according to the intensity of the illumination to which it is exposed

The general system As already stated we know that retinene can readily be reduced to vitamin A outside the eye This reduction has been clearly demonstrated in the intestines There is no evidence however that this mechanism is ever brought into action except in the artificial circumstance of experimental dosing with retinene* It seems reasonable to assume, however, that the existence of the enzyme system could provide protection for vitamin A, in regard to at least one possible direction of oxidation Thus in the event of any form of stress causing a tendency towards the oxidation of the vitamin to its aldehyde a system is present which can facilitate its reversion to the alcohol

Remote effects of vitamin A In the absence of any knowledge of the immediate biochemical action of vitamin A in the general system it may be helpful to review its effects as they appear at long range In Chapters 25, 36 and 38 we have seen that vitamin A encourages the formation of mucus secreting cells which synthesise glycoproteins as opposed to keratinised tissues The general effect is to favour the formation of living secretory cells, at the expense of cells which have stopped living

* Or possibly during the conversion of carotene

in order to provide inert structural components of the body. Disturbances

necessary keratin, which cannot be loosened off by the unhealthy living cells in the deeper layers In both avitaminosis and hypervitaminosis A there is a lack of balance between the bone cells and the intercellular substance

Any conclusion which would narrow down our theories as to the mode of action of vitamin A, within this loose framework, would seem at present unjustified It seems possible, however, that the vitamin may be concerned at some definite point in the synthesis of mucins or glycoproteins Thus it may be necessary for the linkage between protein and glucosamine, or for the introduction into the mucin molecule of the sulphate group In favour of this last proposition evidence was obtained by Fell, Mellanby and Pelc [12] by tissue culture In control experiments the squamous epithelium of presumptive chick ectoderm took up only a little labelled sulphate The mucus secreting epithelium which appeared in response to excess of vitamin A however absorbed the sulphate strongly

Direct influence of vitamin A on tissues

Tissue-culture experiments by Fell and Mellanby (Chap 28) have also been important in regard to rival theories as to whether vitamin A acts directly on affected tissues or indirectly through the production of some unknown substance which is synthesised elsewhere In favour of the indirect theory we know that vitamin A tends to accumulate in the liver, and that it cannot readily be detected in the mucous membranes and bones where its deficiency is most damaging It might be surmised therefore, that some factor necessary for the health of the membranes and bones is synthesised in the liver and is transferred by the blood to other sites Possibly this substance might be the complex between vitamin A and protein which maintains the normal level of vitamin A in the blood, and which is presumably formed by the liver The tissue culture experiments indicate, however, that the change from squamous to mucus secreting cells can be induced simply by adding large amounts of vitamin A directly to the culture medium As a possible criticism which could doubtless easily be discredited by experiments, we may note that the vitamin was added in harmful excess We raise the question, therefore, whether any other toxic substances might have similar effects Experiments on the possibility of changing mucous membranes to squamous epithelium, by their cultivation in a medium containing plasma from animals deficient in vitamin A, would be well worth the trouble that would undoubtedly be involved in overcoming technical difficulties

References p 559

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Independent work by Morton and his colleagues [16, 18] also established the presence of two substances in the livers of deficient rats S A had an absorption band at 272 $m\mu$ and inflections at 330 and 410 $m\mu$ S C had its maxima at 275 $m\mu$ and 330 $m\mu$ with inflections at 233 and 283 $m\mu$ The two substances were present in varying amounts in the livers or other tissues of various animals Thus the richest source of S A was found to be hen liver while human kidneys contained the highest concentration of S C The livers of rats deficient in vitamin A when compared with those of normal rats were slightly richer in S A and very much richer in S C When the rats had been completely deficient in vitamin A the livers contained no less than 90 times more S C than was found in the livers of rats which had been fed upon a stock diet Even livers from rats which had been dosed with vitamin A at the rate of 25 i u daily an adequate dose according to most criteria were found to contain 40 times more S C than those of rats given a stock diet Further evidence was obtained therefore that the metabolism of substances A and C was influenced by the vitamin A status Spectroscopic studies on various other tissues indicated the presence of several other unknown substances

Further developments will be awaited with interest For the present the evidence that the formation of large amounts of S C is a specific indication of vitamin A deficiency seems suggestive but still inconclusive No trials appear to have been made on the effect of starvation or of variations in the intakes of nutrients other than vitamin A on the concentrations of

S C and its congeners It must be difficult, moreover, to estimate accurately either S A or S C in the presence of the large amounts of vitamin A Whatever may be the final outcome of these investigations, in regard to vitamin A metabolism, however, it seems probable that the discovery of a series of new unstable lipoid constituents will have important general repercussions. According to Morton[16] S A has a molecular weight of about 580 two ketone groups and 7 double bonds estimated by perbenzoic titration. It can be reversibly oxidised and reduced

VITAMIN A IN MEDICINE

The vitamin A status of the human subject, in health and in disease, has been discussed at length in Chapters 29–33, and no attempt will be made to provide a detailed summary in this section We may recall, however, that diets which are grossly deficient in vitamin A are known to cause xerophthalmia and keratomalacia in infants, although the appearance of these conditions may be precipitated by other diseases Defective dark adaptation may be detected in deficient adults and there may be hyperkeratosis of the skin Daily doses of 2500 i u of preformed vitamin A, or larger amounts of carotene are officially considered as being sufficient to avert danger of vitamin A deficiency in adults Illness affects the vitamin A status as studied by chemical estimations of the vitamin and the degree of disturbance is much greater in some diseases than in others Symptoms of vitamin A deficiency can sometimes occur as a secondary effect of illness and particularly of conditions which affect the absorption of the vitamin, even when the diet is not grossly inadequate Certain skin diseases in which the vitamin A status seems to be disturbed and others in which there is less evidence of a disturbance, sometimes respond to massive dosing with vitamin A Caution must be exercised against injudicious therapy however, since overdosing can lead to toxic symptoms In chronic overdosing bone abnormalities are a constant feature Single massive doses cause a transient hydrocephalus in infants

Faced with such a range of conditions in which attention to the vitamin A status may be necessary the clinician may appreciate suggestions as to the prescription of vitamin A It may be helpful, therefore, to consider the circumstances in which it seems reasonable to give special supplements of the vitamin The headings given in Table 60 may be supplemented by the notes which follow

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Refere ces p 559

TABLE 60

CIRCUMSTANCES IN WHICH VITAMIN A MAY BE PRESCRIBED

Type of treatment	*Purpose actual or alleged*	*Suggestions for dosing*
Curative treatment	The cure of frank xerophthalmia hemeralopia or hyperkeratosis of dietary origin	2000 i u per kg of body weight daily (see text)
Prophylactic dosing	Alleged promotion of good growth and health Alleged prevention of colds and common infections	To make up total intake of 2500 i u daily for standard adult (See also Table 44 Chap 30)[a]
Rehabilitation therapy	The replacement of stores of vitamin A which have been lost through disease	A total of 300 000 i u during convalescence or treatment (see text)
Treatment of refractory diseases	Treatment of diseases and particularly certain skin diseases in which there appears to be resistance to the absorption or action of vitamin A	200 000 i u daily in adults[b]
Prophylaxis in pregnancy and lactation	Restitution to the mother of vitamin A which is transferred to the foetus or secreted during lactation	To make up to total intakes of 3000 i u daily during pregnancy and 4000 i u during lactation (See also Table 44 Chap 30)[a]
Prophylaxis in old age	Prevention of abnormalities in the eyes and skin	30 000 i u daily[c]

[a] *Doses of up to 10 times the recommended levels of preformed vitamin A should do* no harm but hypervitaminosis is possible with 100 times the recommended levels

[b] Reduce the dose if treatment is prolonged and stop completely if hypervitaminosis A is suspected

[c] According to Kirk and Chieffi [20]

Curative treatment When the patient is suffering from xerophthalmia the need for treatment is urgent and indisputable The clinician will not trouble about the niceties of giving a barely adequate dose but will administer the preformed vitamin as halibut liver oil or as some other concentrated source at the highest level consistent with the avoidance of hypervitaminosis A dosage of 2000 i u per kg of body weight daily should be well tolerated If the eye lesions are associated with a defect in the absorption of fat the vitamin will doubtless be administered in the form of an aqueous emulsion If a child is not eating an aqueous emulsion may be injected The treatment will be continued until the eye lesions have healed as far as possible By this time enough vitamin should have accumulated in the liver to prevent any danger of further deficiency in the immediate future

When a subject who has received a defective diet is suffering from clinical night blindness the same rate of dosing may be recommended A cure should result very promptly unless the hemeralopia is conditioned by some other

abnormality, such as defective absorption of the vitamin It must be remembered, moreover, that deficiency of vitamin A is not the only cause of defective dark adaptation

Preventive dosing If statistics on the consumption of pharmaceutical preparations of vitamin A were available in industrialised countries, such as Great Britain and the U S A they would probably reveal that preventive dosing accounts for at least 90% and possibly verging on 100% of the total supplies

The vitamin is usually taken, often in conjunction with other vitamins with the idea of ensuring normal growth in children and of preventing colds and other infections in subjects of all ages Concrete evidence of the reality of these benefits, at least to an average subject consuming an ordinary diet, is virtually non existent The consumption of the vitamin may therefore be regarded as a form of insurance

Two arguments, suggested by common sense but unproven may be advanced in favour of such insurance Firstly we may consider that since vitamin A can be stored in the liver it is wise to keep the stores at a high level These high stores, although certainly not the only factor concerned should assist in the rapid mobilisation of the vitamin into the blood stream in periods of metabolic stress Secondly we must expect from the examination of livers from cases of accidental death (Chap 29 Fig 23) that the liver reserves in groups of subjects not specially dosed with vitamin A, will show very wide variations In default of any simple method for discriminating during life between subjects having high and low reserves it may seem reasonable to provide supplements of vitamin A for the whole group The vitamin may then reach those who need it while in reasonable doses it will at least do no harm to those who are already adequately supplied

If the taking of special supplements of vitamin A is accepted as a reasonable precaution there is some evidence to support the commonly held idea that dosing is more desirable in winter than in summer Thus we have seen in Chapter 31 that the incidence of cases of acute vitamin A deficiency is much higher in winter and early spring than in summer and autumn

In this connection moreover, it may be remarked that the common cold also has its higher incidence in the winter months and that claims are frequently made in advertisements that vitamin A preparations will afford protection against colds Unfortunately there seems little justification for this conclusion Sutherland [19], for example, found that the dosing of school children with vitamins A and D had no effect on the number or severity of the colds which they caught In the author s own opinion it would seem unreasonable to expect vitamin A to check the formation of mucus once a cold has started The value of vitamin A therapy would be worth investi

References p 559

gation, however, in patients who tend to develop chronic coughs in continuation of colds.

Rehabilitation therapy. We have seen in Chapter 32 that disease can profoundly affect the vitamin A status As a result the reserves which are found at autopsy in subjects who have died from disease tend to be lower than those who have died by accident It seems reasonable, therefore, to provide vitamin A to convalescent subjects in amounts equal to their losses, as calculated for fatal cases of the disease in question From Table 49 (Chap 32) we may estimate that the losses to be expected in various diseases, at the time of the investigation, were as follows

Disease	*Expected loss of vitamin A, total I U*	*Loss as percentage of reserve in health*
Appendicitis	170,000	50
Cancer	170,000	50
Pneumonia	230,000	70
Chronic nephritis	300 000	89

In diseases from which a good recovery has been made it would seem reasonable, as a general routine precaution from which no immediate benefit is expected, to give a course of dosing which will provide the equivalent of half the median liver reserve to be expected in a healthy subject On the basis of later data than those already quoted a total intake of 300,000 I U may be recommended

In chronic or incurable diseases the situation may be complicated by failure in the absorption or metabolism of the vitamin Often this abnormality may have little effect on the progress of the disease, but the possibility of aggravation of the main lesion by concomitant hypovitaminosis A should be borne in mind

Refractory diseases The evidence which justifies the treatment of certain skin diseases with massive doses of vitamin A has been summarised in Chapter 31 In most instances we must conclude that there has been no deficiency of vitamin in the diet, but that the patient has an unusually high requirement for the vitamin This may arise through a defect in absorption, or through a defect in the mechanism by which the vitamin is transported to its site of action We have seen that the diseases which sometimes respond to massive vitamin A therapy include the rare Darier's disease, Devergie's disease and ichthyosis, together perhaps with common acne Daily doses of 200,000 I U may be tried

Pregnancy and lactation The desirability of restoring the vitamin which has been passed from the mother to the child seems obvious The doses of vitamin A should be adjusted so as to bring

the total daily intakes up to 3000 i u during pregnancy and to 4000 i u during lactation as recommended by the British Medical Association (Chap 30 Table 44)

Old age We have mentioned in Chapter 31 the claim of Kirk and Chieffi [20] that high doses of vitamin A are beneficial in old men and women This conclusion is in agreement with the results of experiments on rats in which Sherman [21] [22] found that much larger doses were necessary to sustain a long life than to promote growth at an early age The rate of dosing recommended by Kirk and Chieffi was 30,000 i u daily

Cases with exhausted liver reserves of vitamin A In large surveys of vitamin A reserves as indicated by the examination of specimens of liver obtained at autopsy from diseased human subjects a few cases will usually be found in which the absence of the vitamin complete or almost complete is associated with lesions which might reasonably be attributed to avitaminosis A Thus death may have been due to pneumonia or urinary infections which are common terminations to vitamin A deficiency in experimental animals In these cases it might be argued that death could have been caused by deficiency of the vitamin irrespective of any other factors which were involved In parallel with such cases however others will have died with the same lesions but while still possessing adequate vitamin A reserves Since the same end results occur with or without the vitamin the importance of the apparent avitaminosis in those cases without liver reserves seems problematical The idea of giving the vitamin as an insurance policy however is certainly strengthened

Vitamin A in Animal Husbandry

As explained in Chapter 34 animals at pasture are ensured of adequate supplies of vitamin A in the form of carotene except when the herbage is burnt up by drought and strong sunshine When fresh young pasture is available carotene is ingested in amounts vastly in excess of the requirements for the formation of vitamin A so that the method of its disposal would make an interesting subject for research Animals which do not graze or which are not allowed access to grass present a separate problem and special arrangements should be made for the inclusion of vitamin A in their diets

Bovines can become deficient in vitamin A under farming conditions when they are stall fed and particularly when sugar beet pulp is a major component of their diet The deficiency does not usually cause xerophthalmia but can result in blindness through injury to the optic nerve As already mentioned it appears that this blindness can sometimes be a delayed effect reflecting a past rather than a current period of deficiency

Young calves are born low in vitamin A and receive both the vitamin

References p 559

and antibodies in the colostrum In America a condition known as X disease characterised by hyperkeratosis has been frequently observed The disease is usually caused by chlorinated naphthalenes which find their way into commercial cattle foods A secondary deficiency of vitamin A has been implicated

Sheep tend to have higher liver reserves of vitamin A than are found in bovines There appear to have been no authentic instances of spontaneous vitamin A deficiency under practical conditions

Pigs and poultry require the inclusion of vitamin A in their diets which are usually mainly composed of fodders almost devoid of vitamin A activity If they are allowed to run loose either species may consume enough grass or other green vegetable matter to suffice their requirements Without this precarious source as during confinement to pens with floors of concrete or mud acute deficiency can only too readily develop

Vitamin A in the Future

biochemistry of the vitamin throughout the greater part of the body We are aware of the abnormali

diseases but are unable to

makes a significant contribi

all we know that the livers of the majority of our population contain substantial reserves of vitamin A but are unable to decide with any confidence whether for this reason the majority have any immediate advantage over the minority with low reserves

In the lives of scientists, however, it is usually their privilege to learn secrets that were never known to their fathers and their fate to be denied the knowledge which will in time enlighten their sons The danger to the continuation of this entirely natural process it would seem lies in the growing bulk of the knowledge which must be handed on from one generation to another The author is not so old that his hopes of learning something more of vitamin A have vanished nor so young that the future still seems unlimited He has had to decide therefore between telling the incomplete story which he knows or waiting to tell a more complete story which he may never know Having chosen the first alternative he hopes that this book will ease the task of collecting scattered knowledge both for his contemporary colleagues and for those to follow He will be happy if it can be of some little service in the planning of future research

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APPENDIX

MISCELLANEOUS INFORMATION

Current

M.W. of vitamin A, $C_{20}H_{30}O$ = 286
M.W. of vitamin A acetate, $C_{22}H_{32}O_2$ = 328
M.W. of carotene, $C_{40}H_{56}$ = 536
International unit (i.u.) of carotene = 0.6 μg
International unit of vitamin A = 0.3 μg vitamin A
= 0.344 μg vitamin A acetate
1 i.u. vitamin A = 1 i.u. carotene (in rat)
$E^{1\%}_{1cm}$, 328 mμ for vitamin A (in propanol) = 1750
Conversion factor (1950) for changing $E^{1\%}_{1cm}$, 328 mμ into i.u. per g (applicable to spectroscopically pure sources only) = 1900
Conversion factor (1934) for slightly impure forms of vitamin A = 1600
$E^{1\%}_{1cm}$, 620 mμ for vitamin A in $SbCl_3$ reaction = 5070
Conversion factor between $E^{1\%}_{1cm}$ at 620 mμ and i.u. per g = 657
$E^{1\%}_{1cm}$, 455 mμ for carotene (in cyclohexane) = 2450
Conversion factor between $E^{1\%}_{1cm}$, 455 mμ and i.u. per g = 678
Daily vitamin A requirement of human adult when supplied as preformed vitamin = 2500 i.u.
Daily vitamin A requirement of human adult when supplied as carotene = 7500 i.u.
Approximate reduction factor between i.u. of carotene and i.u. of preformed vitamin A in supplying requirement of human = 3 (but varies according to source of carotene).

Historical

United States Pharmacopoeia (U.S.P.) unit = international unit. (But until the introduction of vitamin A acetate as standard there were some periods when the U.S.P. unit was slightly smaller than the international standard, owing to the instability of cod-liver oil which was used as a secondary standard.)

Blue value (Rosenheim) = intensity of colour measured by Lovibond standard glasses produced by treating 0 040 g (or ml) of oil made up to 0 20 ml in chloroform with 2 ml of antimony trichloride reagent

Blue unit (Moore) = colour contained in 1 ml of the $SbCl_3$ vitamin A reaction mixture viewed in a layer 1 cm thick when the Lovibond blue reading is 1

An oil having a Blue value (Rosenheim) of 1 contains about 55 B U (Moore) per g

B U (Moore) per mg — 18 2 = Blue value (Rosenheim)

1 B U (Moore) ca 0 6 i u

1 Lovibond unit (Wolff) = 11 Blue units (Moore)

The Sherman unit (*J Amer chem Soc* 47 (1925) 1639) was the daily dose of vitamin A required to produce a standard growth response in rats It was later reported by different workers to be equivalent to 0 5 1 5 i u

VITAMIN A DEFICIENT DIETS FOR EXPERIMENTAL ANIMALS

(CHAPTER 5)

Diet for rats at Dunn Nutritional Laboratory Cambridge The basal diet used for routine vitamin A tests has the following composition

Casein (vitamin free)	20 parts
Sucrose	60
Arachis oil	15
Brewer s yeast (dried)	10
Salt mixture	5

In this diet the vitamin B complex is supplied by the dried yeast Other vitamins are provided in weekly oral doses dissolved in one or two drops of arachis oil as follows

Vitamin E as *dl* α tocopheryl acetate 2 mg per rat weekly

Vitamin K as 2 methyl 1 4 naphthoquinone 50 μg weekly,

Vitamin D as ergocalciferol 1 5 μg weekly

The composition of the salt mixture is as follows

Calcium phosphate $Ca_3(PO_4)_2$	43 5 parts
Potassium chloride	27 2
Acid sodium phosphate anhydrous	11 4
Magnesium sulphate	8 7
Sodium chloride	5 4
Iron citrate	3 8
Potassium iodide	0 1
Manganese sulphate	0 22
Sodium fluoride	0 004

The basal diet and tap water are given *ad libitum*

Diet used by Dr K H Coward[1] During her work as head of the Nutrition Department of the Pharmaceutical Society of Great Britain Miss Coward fed her rats upon the diet described below It will be noticed that the fat component was omitted

Casein	15%
Dextrinised starch	73
Brewer s yeast (dried)	8
Salt mixture (Steenbock s No 40)	4

References p 569

Vitamin D was supplied as ergocalciferol at the rate of 8–10 i.u. (0.2–0.25 μg) per rat weekly, and vitamin E as 1 mg of *dl*-α-tocopheryl acetate.

Steenbock's salt mixture No. 40 is made up as follows:

NaCl	9.49%
$MgSO_4, 7H_2O$	10.00
$Na_2HPO_4, 12H_2O$	14.59
K_2HPO_4	28.28
$CaHPO_4$	28.29
Ca lactate, $5H_2O$	6.26
Fe citrate, $6H_2O$	2.44
KI	0.65

American modification of Coward's diet.

Ellenberger *et al.*[2] used a modification of Coward's diet in extensive assays in connection with work by the United States Pharmocopoeia [3] on the setting up of vitamin A acetate as a standard for vitamin A. Casein was increased to 18% and 5% of fat was introduced in the form of hydrogenated vegetable oil, both changes being balanced by reductions in the percentage of dextrinised starch.

Vitamin D was provided by irradiation of the yeast, and vitamin E was presumably supplied by the vegetable oil.

Casein (hot alcohol extracted)	18%
Corn starch (dextrinised)	65
Dried yeast (irradiated)	8
Vegetable oil (hydrogenated)	5
Salt mixture	4

Vitamin A low diet for chickens.

Castano *et al.*[4] have used the following diet, which presumably contains traces of carotene, for experiments on chicks.

Ground wheat	35.75%
Ground oats	10
Wheat bran	10
Wheat standard middlings	10
Soya bean meal (expeller processed)	10
Dried whey	6
Commercial casein	6
Dried brewer's yeast	6
Pulverised limestone	2.5

Steamed bone meal	1 3
Iodised salt	0 3
Manganese sulphate	0 05
Activated animal sterol (vitamin D)	0 1
Cottonseed oil	2

Purified vitamin A deficient diet for turkeys

Van Reen *et al* [5] compounded a more elaborate diet which was virtually free from even traces of vitamin A for use in experiments on the requirements of turkey poults for the vitamin

Casein (alcohol extracted)	36 7%
Gelatin	10 5
Starch	27 2
Sucrose	10 0
Maize oil	4 0
Cellulose	2 0
Salt mixture	5 0
$CaHPO_4 \cdot 2H_2O$	2 0
$CaCO_3$	2 0
Choline chloride	0 2
Inositol	0 1
para Aminobenzoic acid	0 1

Supplements of the following vitamins were also supplied in mg per 100 g of the diet

Thiamine hydrochloride	1 0 mg
Pyridoxine hydrochloride	1 0
Riboflavin	2 0
Calcium pantothenate	2 0
Nicotinamide	5 0
Menadione (vitamin K)	2 5
Mixed tocopherols	2 5
Biotin	0 04
Folic acid	0 2

Vitamin D was given as activated animal sterol at the rate of 280 units per 100 g of diet

The composition of the salt mixture was as follows

$CaCO_3$	21 5%
$CaHPO_4 \cdot 2H_2O$	33 6
$MgCO_3$	2 01

References p 569

$MgSO_4, 7H_2O$	2 42
NaCl	10 25
KCl	1.69
Ferric ammonium citrate	2 39
KH_2PO_4	25 4
$MnSO_4, 4H_2O$	0 403
$CuSO_4, 5H_2O$	0 129
$Al_2(SO_4)_3K_2SO_4$ $24H_2O$	0 019
KI	0 048
$CoCl_2, 6H_2O$	0 0048
$ZnSO_4, 7H_2O$	0 040
NaF	0 081

The above diet could doubtless be modified, by changes including the replacement of the gelatin and part of the casein by carbohydrate, for use as a purified diet for rats

Diet for cattle Diets for the study of vitamin A deficiency in cattle, more than 3 months old, have usually been based upon sugar beet pulp The following diet was found effective by Guilbert and Hart •

Dried molasses (sugar beet) pulp	70%
Rolled barley	14
Cottonseed meal	15
$CaCO_3$	1

Diets of this type may be low in protein, and other nutrients, besides being deficient in vitamin A Other components, such as linseed cake and oat chaff and straw, may be introduced if available

For young calves diets may be based on efficiently skimmed fresh milk, or on dried skimmed milk To preserve the caloric intake the butter fat should be replaced by an inactive vegetable oil, which must be carefully emulsified with the skimmed milk

Preliminary preparation of experimental animals The importance of preventing animals which are intended for the production of vitamin A deficiency from accumulating large stores of the vitamin during early life has already been mentioned (Chap 5) In rats the amounts of vitamin which are transferred to the foetuses during pregnancy are usually small, provided that the mother has not received rich concentrates (Chap 21) Even during lactation the amount of vitamin passing to the young may not be sufficient to make the animals completely useless for experimentation

If the young are allowed to eat a mixed stock diet for themselves for only a few days, however, the subsequent depletion may be delayed for many months In practice it may be convenient to allow the mother her normal stock or cube diet, but without liver or cod-liver oil during pregnancy and for the first 14 days of lactation A diet low in vitamin A based for example on skimmed milk and cereals, may then be substituted The young may continue to receive it, after weaning, until they are ready for restriction to the completely deficient diet

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DETERMINATION OF CAROTENE IN BIOLOGICAL MATERIALS

(CHAPTERS 6 AND 30)

(Kindly contributed by DR V H BOOTH[1])

General procedure

A heavy-gauge 50 ml beaker is weighed first empty and then with the sample A quantity of quartz powder roughly equivalent to the weight of the sample is added followed by about 10 ml of a 1 1 mixture of light petroleum and acetone saturated with quinol The sample is ground under this solvent with the use of flat-bottomed glass pestle The coloured supernatant solution is decanted into a separating funnel that already contains water. The grinding with solvent and decanting is repeated until no more pigment can be extracted The acetone is washed from the extract with water, preferably by attaching a simple continuous drip apparatus to the separating funnel (Fig 47)

All the carotenoids and chlorophylls are now concentrated in the light petroleum layer The lower layer of water is discarded The solution of pigments is decanted into a chromatographic column of alumina-Na_2SO_4 mixture about 2 cm diameter by 4 cm high, or longer if a rich sample has been extracted Percolation is aided by suction If all is well—the adsorbent in good condition, no acetone remaining in the extract—the pigments will be adsorbed on the alumina mixture and the filtrate will be colourless When 2% acetone in light petroleum is drawn through the column, a narrow orange band of carotene separates from the other pigments and passes down the column, leaving other carotenoids and chlorophylls still adsorbed

The eluate is collected in a measuring vessel and its volume is recorded The extinction (or optical density) is observed at 450 mμ in a spectrophotometer, or in an absorptiometer with a blue filter

Notes

In green leaves there is an enzymic system that destroys carotene when the leaves are damaged Therefore there must be no delay between preparing a sample and extracting it

Size of sample to be extracted

In order to complete the extraction quickly and to keep volumes of extracts easily manageable it is worth taking trouble to use very small samples, e g less than 1 gram

By skilful use of sharp knives and scissors this can usually be done However some materials compel the use of larger samples If these contain much water they are best extracted with a solvent mixture richer in acetone but in this event the extracts will have to be filtered during transfer into the separating funnel Although acetone is a better extractant it does not encourage particles to settle as petrol does

Large samples require a mechanical aid for their disintegration The Waring blendor and its imitators are often used for this purpose It should be understood that while large samples need a blendor extracts from small samples are more quickly prepared for chromatography by the beaker method

Special materials The method briefly outlined above can be applied to many plant and some animal tissues but modifications are advisable for some materials Dehydrated materials require soaking with water before extracting Fatty materials may need to be saponified because fat in large amount interferes with chromatography The carotenoids of carrot roots are mostly carotene hence determination of total carotenoids may be adequate thereby saving washing and chromatography

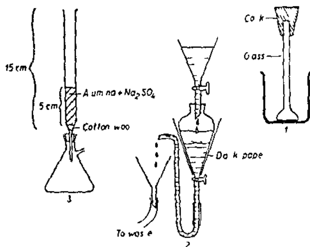


Fig 47 Apparatus recommended by Booth for the estimation of carotene (1) Beaker and pestle for the preliminary extraction (2) Automatic washing device for the removal of acetone from the extract (3) Alumina adsorption column for the separation of carotene from other pigments

The adsorbent Many adsorbents have been described and some are in regular use Magnesia has good points although alumina betters it in speed Alumina takes up moisture from the air and after the bottle has been opened a few times the powder will no longer hold carotene This deterioration is prevented by mixing the alumina with about equal

weight of fine crystal anhydrous sodium sulphate If the alumina has already taken up moisture it should be heated at 150° for 12 hours or so The sulphate may require the same treatment

If the alumina is too moist carotene may not be held but will appear in the eluate before the 2% acetone is applied In the unlikely event that the alumina is too retentive carotene will not be satisfactorily eluted with 2% acetone 3 or 4% may be necessary

TABLE 61

CAROTENE CONTENTS OF SOME VEGETABLE MATERIALS BASED ON FRESH WEIGHT (ESTIMATED CHEMICALLY BY DR V H BOOTH)

	Samples	*Carotene i u /100 g*	*s d* *	*Portion*
Bean French (*Phaseolus vulgaris*)	6	930		whole pods
Bean runner (*Phaseolus coccineus*)	9	650	180	whole pods
Blackcurrant (*Ribes nigrum*)	7	250		fruit
Brussels sprouts (*Brassica oleracea* var *gemmifera*)	20	700	250	sprouts
Carrot root (*Daucus carota*)		20 000		mature
Carrot leaf	149	19 500	3 300	
Clover (*Trifolium repens*)	37	23 000	3 100	leaves
Cress (*Lepidium sativum*)	20	8 300	1 600	leaves
Gooseberry (*Ribes grossularia*)	7	230		fruit
Grasses various pasture	46	19 500	3 800	leaves
Lettuce (*Lactuca serriola*)	26	2 600	2 100	
Mint (*Mentha spicata*)	9	19 000	5 700	
Narcissus selected cultivars	10	500 000		crowns**
Nettle (*Urtica dioica*)	10	23 000	4 000	
Parsley (*Carum petroselinum*)	12	13 800	2 500	
Pea (*Pisum sativum*)	9	670	130	
Spinach beet (*Spinacia oleracea*)	14	11 000	2 000	leaves
Tomato (*Solanum lycopersicum*)	23	1 300	350	
Watercress (*Nasturtium officinale*)	14	5 200	2 100	

* Standard deviation of a single sample

** The remarkably high carotene contents of this material explains the results of early biological tests by Coward [2]

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1 V H BOOTH *Carotene its Determination in Biological Materials* W Heffer & Sons Ltd Cambridge 1957
2 K H COWARD *Biochem J* 17 (1923) 145

ESTIMATION OF VITAMIN A BY ULTRAVIOLET SPECTROPHOTOMETRY

(CHAPTER 6)

The high accuracy of this technique when applied to concentrated sources of the vitamin, and the difficulties in its application to sources of low potency have been mentioned in the main text Practical details for the preparation and examination of specimens, and for the choice of a suitable spectrophotometer and glassware, have been given by the Association of Vitamin Chemists [1]

Correction procedure of Morton and Stubbs [2-4] This useful method for deducting irrelevant absorption, in the measurement of vitamin A in spectroscopically impure sources is based on an exact knowledge of the absorption curve of the vitamin in the particular solvent which is being used A measurement of absorption is first made on a solution of the material under investigation at the wave length which is known to be the maximum for pure vitamin A irrespective of whether this is the actual maximum for the impure spectrum or not This observation gives what we may call the gross reading for vitamin A

In order to find the actual or net amount of vitamin measurements are then made at two other chosen wave lengths ("fixation points") one on each side of the maximum The deduction that is necessary for irrelevant absorption may then be calculated from the degree of "flattening" of the observed adsorption curve as compared with that of the pure vitamin The flattening is measured in general principle by the amounts by which the absorption at the two side wave lengths exceed those which would be expected for the pure vitamin

The assumption is made, which must be generally true that the absorption curve of the substances responsible for irrelevant absorption will approximate to a straight line, at least over the limited range between the two side wave lengths at which observations are made This line may be horizontal, may slope upwards towards the longer wave lengths or may slope upwards towards the shorter wave lengths The last possibility will occur most frequently, and our attention may first be confined to sources of the vitamin in which the irrelevant absorption follows this pattern

According to the Morton-Stubbs procedure the two side observation wave lengths are so chosen that the absorption of pure vitamin A at each position would be exactly equal In practice it is convenient to select the two wave

References p 576

weight of fine crystal anhydrous sodium sulphate If the alumina has already taken up moisture, it should be heated at 150° for 12 hours or so The sulphate may require the same treatment

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In order to find the actual or net amount of vitamin measurements are then made at two other chosen wave lengths (fixation points) one on each side of the maximum The deduction that is necessary for irrelevant absorp tion may then be calculated from the degree of flattening of the observed adsorption curve as compared with that of the pure vitamin The flattening is measured in general principle by the amounts by which the absorption at the two side wave lengths exceed those which would be expected for the pure vitamin

The assumption is made which must be generally true that the absorp tion curve of the substances responsible for irrelevant absorption will approx imate to a straight line at least over the limited range between the two side wave lengths at which observations are made This line may be horizontal may slope upwards towards the longer wave lengths or may slope upwards towards the shorter wave lengths The last possibility will occur most fre quently and our attention may first be confined to sources of the vitamin in which the irrelevant absorption follows this pattern

According to the Morton Stubbs procedure the two side observation wave lengths are so chosen that the absorption of pure vitamin A at each positic would be exactly equal In practice it is convenient to select the two wav

References p 576

lengths at which the absorption of the pure vitamin would have fallen to 6/7 of its value at the maximum.

In these circumstances it is clear that any increase of the observed absorption at the short wave side over that at the long wave side will be due to the upward slant of the absorption line of the irrelevant absorption. The correction procedure takes place in two stages.

Stage 1. A deduction is made from the observed extinction at the absorption maximum of vitamin A which is related to the degree of sloping of the irrelevant absorption line.

Stage 2. A further reduction is made to allow for the intensity of the irrelevant absorption at the lowest point of the slope in the region under study.

Example. The method may perhaps be more simply explained, after these preliminary notes, by taking an actual example, as given by Morton and Stubbs.

A specimen of cod-liver oil was dissolved in cyclohexane, for which solvent the three "fixation points", found by measurements on pure vitamin A, are 313, 328 and 338 5 mμ. The following data were obtained (see also Fig. 48):

mμ	E
313	0.640
328	0.712
338.5	0.620

Stage 1. The total slope between 313 and 338.5 mμ is E 0.640 – E 0.620 = E 0.020. *The slope correction at the maximum for vitamin A* (328 mμ) will obviously be proportional to the distance of this position along the wavelength axis.

The correction for slope at 328 mμ is therefore

$$\frac{\text{E } 0.020 \times (338.5 - 328)}{(338.5 - 313)} = \frac{\text{E } 0.020 \times 10.5}{25.5} = 0.008$$

Stage 2. After deducting this "slope" correction the absorption at 328 mμ becomes 0.704. The absorptions at this wave length, and also at 338.5 mμ, will now each include the same "flat" irrelevant absorption, which we may call x. We know also, from the positions at which the fixation points have been chosen, that after x has been deducted the absorption at 328 mμ will be 7/6 of that at 338.5 mμ. We have therefore the equation

$$\frac{0.704 - x}{0.620 - x} = 7/6$$

$$x = 0.116$$

The absorption at 328 mμ due to vitamin A is therefore 0 704 − 0 116 = 0 588 or

$E^{1\%}_{1cm}$ 328 mμ (0 712 gross) = 0 588 (corrected)

whence i u per g = 0 588 × 1900 = 1120

It may be pointed out that before spectrophotometric examination much of the irrelevant absorption is usually removed from cod liver oil and other relatively weak sources of the vitamin by saponification

Abnormal types of irrelevant absorption If E at 338 5 mμ exceeds E at 313 mμ the slope correction can readily be calculated in a reverse direction If the observed absorptions at the two positions should be identical stage 1 of the correction can be omitted

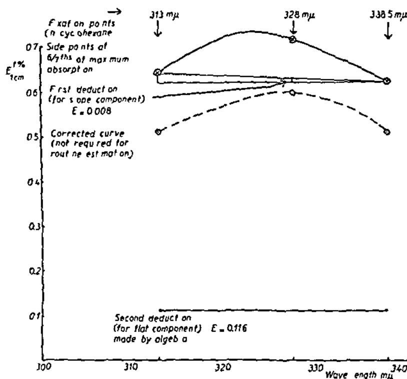


Fig 48 Estimation of vitamin A by ultraviolet spectrophotometry The correction procedure of Morton and Stubbs The actual readings are marked with crossed circles

Correction formulae Recently the two stage method of Morton and Stubbs has been largely superseded by the use of standardised equations which are based at least partially on the same principles According to the Association of Vitamin Chemists [1] the following formula applies when *iso*propanol is the solvent The change in fixation points between cyclohexane and *iso*propanol should be noted

References p 576

$$E\ (\text{corrected}) = (7 \times E_{325\,m\mu}) - (2.625 \times E_{310}) - (4.375 \times E_{334})$$

Korr[5] has suggested that this equation may be applied more easily if it is rewritten as

$$E\ (\text{corrected}) = 7(E_{325} - E_{310}) + 4.375\,(E_{310} - E_{334}).$$

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ESTIMATION OF VITAMIN A IN FATS BY THE ANTIMONY TRICHLORIDE METHOD

(CHAPTER 7)

As usually applied the antimony trichloride method gives less exact results than those obtained by ultraviolet spectrophotometry, at least as judged by the spread of repeated readings on the same material On the whole, however, the antimony trichloride method is the more "foolproof", since systematically high values can be found by ultraviolet measurements if due precautions are not taken for discounting irrelevant absorption The danger with antimony trichloride, conversely, is that unduly low results may be obtained through the presence of substances which interfere with the full development of blue colour Since the antimony trichloride method requires less costly apparatus than is necessary for the ultraviolet method it may be the choice of workers, with limited technical resources, who desire to investigate points concerning vitamin A without becoming specialists in the field.

Saponification With halibut liver oil and other potent sources, the interference by the lipoids in the development of the blue colour by vitamin A may be small It is even possible that the reduction in blue colour may be less than that which could be caused, at least in inexpert hands, by a small loss of vitamin A during the removal of the lipoids by saponification With cod liver oil, however, the formation of blue colour may be doubled by saponification With butter the colour may be increased by 10–20 fold

Many workers greatly overestimate the time which is necessary for saponification With most oils it is only necessary to shake up the oil, or melted fat, with boiling alcoholic potash for the process to be completed almost immediately

Standard procedure Each gram of oil requires 0 44 ml of an aqueous solution of KOH (made up by dissolving 40 g of KOH in 27 ml of distilled water) and 2 ml of ethanol (97%) The KOH solution and alcohol are mixed together, brought to the boil, and poured on to the oil. During this process the flask is agitated and may be partially immersed in a hot water bath Saponification will be indicated by the contents of the flask becoming homogeneous, and should be complete within 2 minutes

Distilled water (10 ml per g of fat) is then added and the mixture is extracted, usually in a separating funnel, with peroxide-free ether (20 ml per g of fat) Undue delay after adding the water should be avoided, as the solu-

References p 581

tion will become turgid on standing The aqueous layer is discarded or is removed and is extracted for a second time with ether (In practice the removal of vitamin by the first extraction is reasonably complete but further extracts may be given if high accuracy is required)

The ether extracts are combined and are first shaken vigorously with a small volume of distilled water then more cautiously with larger volumes (A continuous washing device as used by Booth in carotene estimations (page 571) could probably be used with advantage) The ether layer is next dried by passage through anhydrous sodium sulphate contained in a sintered glass funnel and is evaporated to dryness under diminished pressure (Fig 49)

Alternative procedure When the oil is fairly rich in vitamin A (e g cod liver oil) quite a small quantity will provide enough vitamin for the antimony trichloride test In these circumstances it may be convenient to keep the aqueous phase as small as possible and to ensure a virtually complete extraction of the vitamin by the use of a single large volume of ether

500 mg of oil is weighed out into a 5 ml flask KOH solution (0 22 ml) and ethanol (1 ml) are added and the flask is heated on a water bath for 1 minute with shaking The contents of the flask are poured into a separating funnel containing 50 ml of ether The flask is then washed four times with 2 ml of distilled water each time the washings being transferred to the separating funnel The funnel is vigorously shaken and is then left until the layers have separated when the aqueous layer is discarded The ethereal layer is washed carefully with 25 ml of water which is discarded and is filtered through anhydrous sodium sulphate into a flask of about 100 ml capacity suitable for evaporation under diminished pressure

The antimony trichloride reagent The strength originally recommended for this reagent was 30% Somewhat less concentrated solutions however have the advantage that they are less liable to deposit crystals on standing As supplied by Messrs British Drug Houses Ltd the reagent has the analysis of 8 85% w/w of Sb_2O_3 and 86 1% w/w of $CHCl_3$ These figures correspond to about 21% of the trichloride w/v Even at this concentration the reagent should not be kept in cold weather in an inadequately heated store room

Colour production The concentrated source of vitamin A or the residue obtained after saponification is dissolved in a convenient volume of chloroform Portions of this solution are taken by a blood pipette for treatment with antimony trichloride Sometimes the vitamin may be placed in the cell first and the reagent added but the reverse process seems best for small quantities of vitamin In either case the density of colour must

fall within a prescribed range If the colour is too weak, and more particularly if it is too dense, the reading must be discarded and repeated with a more appropriate volume of the chloroform solution A check must also be made that the reading is actually due to the formation of a blue colour, and not to a different colour or to turbidity The danger of accepting erroneously high readings is obviously much greater, of course, with modern photoelectric apparatus than with old fashioned visual apparatus Two illustrative examples may be given

Lovibond tintometer 0 90 g of cod-liver oil is saponified, and the non saponifiable residue is dissolved in 20 ml of chloroform 0 10 ml of this solution is taken, one drop of acetic anhydride is added (as a precaution against turbidity) and the volume of the mixture is made up to 0 5 ml with chloroform The antimony trichloride reagent 2 ml is added by

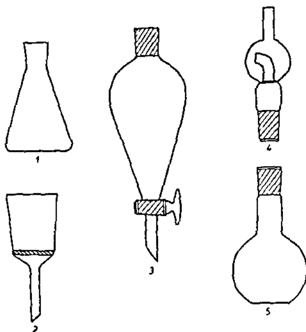


Fig 49 Apparatus used during the saponification of fats containing vitamin A or during its extraction from liver or other materials (1) Conical flask for alkali digestion (2) Sintered glass funnel (3) Separating funnel (4) Trap for evaporating flask The extract cannot be contaminated by a blow back from the rubber tube of the suction pump which will have been used for previous estimations Care must be taken however that the extract under investigation does not bubble over into the trap (5) Evaporating flask The extract at the start of evaporation should not occupy more than half its volume

means of an automatic pipette (Fig 50), and the colour is matched rapidly by combinations of graded blue and yellow glasses After repeated tests, with the glasses already adjusted have confirmed a correct match the calculation is made as follows

References p 581

Volume used = 0 10 ml

Reading = 5 0 blue 2 yellow (neglected)

$$\text{I.U. per g} = 5 \times 2\,5 \text{ (vol in cell)} \times \frac{20 \text{ ml}}{0\,1 \text{ ml}} \times \frac{1 \text{ g}}{0\,9 \text{ g}} \times 0\,6 \text{ (conversion factor)}$$
$$= 1670 \text{ I.U.}$$

Photoelectric absorptiometer A drop of halibut liver oil weighing 18 5 mg is dissolved in 10 ml of chloroform and 1 ml of this solution is made up to a total volume of 25 ml thus making a final dilution to 250 ml A cell is charged with 2 ml of the antimony trichloride reagent containing a little acetic anhydride freshly added (2 5% v/v) and is placed ready in the absorptiometer 0 3 ml of the vitamin solution is taken

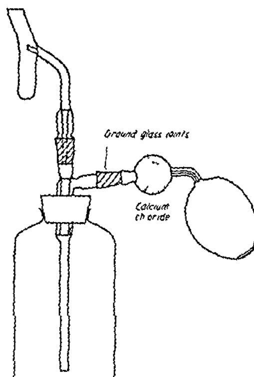


Fig 50 Automatic pipette designed and supplied by Messrs British Drug Houses Ltd for delivering 2 ml of the antimony trichloride reagent By pressing the bulb the reagent is pumped up into the pipette The excess flows back when pumping is stopped The pipette is removable being attached to the pumping part of the apparatus by a ground glass joint (Care must be taken to avoid too forceful pressure of the bulb which may cause premature detachment of the pipette and its breakage by falling It is usually advisable to hold the pipette lightly in place until pumping is completed)

and run rapidly into the cell which is quickly stirred with a small glass stirrer After withdrawing the stirrer and allowing a second or two for the galvanometer to become steady a reading is taken Shunting of the galvanometer to reduce its fluctuations facilitates reading On reference to a calibration curve it is found that the galvanometer deflection was equivalent

to 0 54 I u per ml of the reaction mixture The concentration of vitamin A in the oil was therefore as follows

$$\text{I u per g} = 0\ 54 \times 2\ 3\ (\text{vol reaction mixture}) \times \frac{1000\ \text{mg}}{18\ 5\ \text{mg}} \times \frac{250\ \text{ml}}{0\ 3\ \text{ml}}$$

$$= 56\ 000$$

With photoelectric spectrophotometers such as the Beckman and the Unicam it is unnecessary for workers to make their own calibration curves Results may be worked out on the basis of the known extinction for pure vitamin A[1]

$$E^{1\%}_{1\text{cm}} \text{ at } 620\ \text{m}\mu = 5070$$

Even with apparatus of this type, however observations on a solution of pure vitamin A will provide a useful check on the correctness of the workers technique and calculations

Caution In all work with the antimony trichloride reagent precautions must be taken against the production of turbidity in the reaction mixture caused by moisture and against the formation of white films of oxychloride on glass surfaces This filming can be caused not only by contact with the reagent but also by fumes The film may readily be removed by concentrated hydrochloric acid

SEPARATION OF VITAMIN A ALCOHOL FROM ESTERS

When information is required on the extent to which vitamin A is esterified chromatographic methods of separation may be applied The esters are adsorbed much less strongly than the free alcohol on columns of bone meal[2] or alumina[3] Paper chromatography with a mixture of *iso*propyl alcohol and water as the developing solvent has also been advocated[4]

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ESTIMATION OF VITAMIN A IN LIVER

(CHAPTERS 7, 29 32)

Digestion with alkali. If the quantity of the specimen is not a limiting factor about 5 g of the material is collected, preferably in several pieces taken from different lobes, and is weighed to within 0 1 g It is convenient to use a small beaker of 50 ml capacity for the weighing 10 ml of 5% aqueous KOH (or NaOH) is added and the liver is chopped fairly finely with scissors The material is transferred to a 50 ml conical flask which is loosely corked and placed in a hot water drying oven until the tissues have been digested

Extraction of fat After cooling the digest is transferred to a separating funnel of 100 ml capacity, which may for convenience be graduated Ethanol (5 ml) is added and the mixture is shaken This has the effect of "loosening' the fat Peroxide-free ether (50 ml) is then added, and is shaken strongly with the digest for at least a minute The layers are allowed to separate, and the aqueous layer is run off According to the degree of accuracy which is required this layer is now discarded, or is preserved for further extractions If the former course is taken an addition of 10% may be made to the final value in compensation for the small fraction of vitamin which has been discarded The ethereal extract is washed first by shaking strongly with 5 ml of water, and then more gently with 50 ml of water The extract is dried by filtering through a 2 cm layer of anhydrous sodium sulphate, contained in a sintered glass funnel, and is evaporated to dryness on a hot water bath under diminished pressure

Saponification The above method of extraction is not expected to saponify the liver fat With most livers the ratio of vitamin A to fat is so high that the interference by fat in the antimony trichloride reaction may be negligible If the fat contents of the liver are unusually high, however saponification may be necessary, and may be carried out according to the methods which have already been described This necessity may well arise in the examination of livers showing fatty infiltration

Colour development The antimony trichloride method is applied in the same way as already described for the estimation of vitamin A in fat A specimen calculation follows

4 9 g of liver was digested and extracted, and the fat was dissolved in chloroform By successive dilutions a concentration was obtained which was

equivalent to that which would have been reached by dissolving the whole of the fat in 500 ml

0 3 ml of this solution gave a galvanometer deflection corresponding to 0 45 I u per ml of the reaction mixture

$$\text{I u per g of liver} = 0\ 45 \times 2\ 3\ (\text{vol in cell}) \times \frac{500\ \text{ml}}{0\ 3\ \text{ml}} \times \frac{1\ \text{g}}{4\ 9\ \text{g}}$$

$$= 352\ \text{I u}$$

Possible modifications When applied to most specimens of liver from adult animals the above method provides much more vitamin than is needed for numerous repetitions of the colour reaction In examining such material therefore much smaller specimens will suffice By taking only a fraction of a gram of liver and a correspondingly small amount of alkali but without reducing the ether in the same ratio very easy and complete extraction of the vitamin may be achieved Estimations should even be possible on scraps of liver such as are obtainable by aspiration technique of biopsy [2] With very small specimens of course efficient sampling may become an important problem

If it is desired on the other hand to examine materials which are low in the vitamin such as normal kidney or the liver from cases of chronic nephritis the standard procedure with 5 g of material may be preferred

Table 62 (pp 584 585) summarizes results obtained by the author [3] on a large number of specimens of human liver collected at autopsy

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TABLE 62

VITAMIN A IN THE HUMAN LIVER IN HEALTH AND DISEASE (MOORE [3])

	No of cases	Cause of death	Age group	Mean age	Vitamin A reserve (i u /g) Means of lowest, middle and highest thirds of group L	M (median)	H	General mean
Group 1, accidental death	40	Death within 7 days of injury	15–59	33	75	220	590	290
	16	,, ,, ,,	Over 60	67	18	100	330	150
	73	Death at any time after injury*	All ages	37	41	150	480	220
Group 2, poisoning	13	Death within 7 days from poisoning	15–59	39	94	170	360	210
Group 3, alimentary tract diseases	43	Gastric and duodenal ulceration	15–59	45	40	110	300	150
	9	,, ,, ,,	Over 60	64	43	110	240	130
	19	Appendicitis	15–59	37	28	110	490	210
	11	Enteritis and colitis	15–59	38	9	74	390	160
	12	Peritonitis	15–59	34	31	75	220	110
	10	Hernia, intestinal obstructions, faecal fistulas	15–59	40	90	160	470	230
	7	,, ,, ,,	Over 60	69	34	95	300	140
	13	Gall bladder diseases	15–59	48	49	110	250	130
	7	,, ,, ,,	Over 60	68	90	100	280	150
Group 4, respiratory diseases	22	Pneumonia (lobar and broncho)	15–59	36	5	63	200	88
	12	Empyema	15–59	37	9	60	240	100
	12	Bronchiectasis chronic bronchitis	15–59	40	18	82	250	120
Group 5, endocrine diseases	15	Diabetes	15–59	42	89	300	870	520
	8	,,	Over 60	66	240	540	750	520
	9	Thyroid diseases	15–59	44	90	310	530	310
Group 6, heart and circulatory diseases	56	Valvular diseases of the heart	15–59	37	7	60	230	96
	26	Cerebral haemorrhage resulting from high blood pressure	15–59	47	25	120	370	170

	20	Coronary diseases	15 59	51	28	100	320	140
	14		Over 60	67	25	76	230	100
Group 7 tuberculosis	36	Tuberculosis all forms	15 59	31	24	96	380	170
Group 8 cancer	76	Cancer all *forms*	15 59	46	43	110	320	160
	36		Over 60	67	23	110	210	110
Group 9 syphilitic diseases	27	Syphilitic aortitis aortic aneurisms peri arteritis nodosa syphilitic cirrhosis	15–59	48	23	95	390	170
Group 10 diseases of the kidney and urinary tract	48	Chronic nephritis	15–59	42	5	25	150	59
	10		Over 60	66	6	23	120	47
	12	Sub acute nephritis	15 59	35	9	75	350	140
	13	Kidney and bladder infections	15 59	39	3	19	320	110
	23	Prostate diseases	All ages	64	7	40	230	86
Group 11 septic diseases	52	Head and spine *infections* otitis antrum and sinus infections cerebral abscesses mastoiditis meningitis	15–59	31	10	73	260	110
	35	Other infections abscesses septicaemia osteomyelitis septic arthritis	15–59	36	9	51	270	110
	33	*Septic ulcerative* malignant subacute endocarditis	15–59	37	22	90	250	120
Group 12 diseases of blood generation etc	31	Anaemia leucaemia purpura haemorrhagica Hodgkin s disease pernicious anaemia Banti s disease	15–59	38	19	130	520	220
Group 13 thrombosis and embolism	23	Thrombosis and embolism	15–59	47	13	89	240	110
Group 14 nerve and spinal cord diseases	13	All types of nerve degeneration	15–59	36	22	110	560	220
Group 15 diseases of women	15	*Hysterectomy* abortion puerperal septicaemia etc	15–59	32	29	96	270	130

* *Including the 56 cases given above*

The same volume of alcoholic potash (*1 N* KOH in 90% ethanol) is then added, and mixing is effected by touching the tube against a whirling nail which is rotated in a motor drill The tube is immersed in a water bath at 60° C for 20 minutes is cooled, and 0 060 ml of a mixture of equal parts of kerosene and xylene is added The contents of the tube are again mixed by touching against the whirling nail and are then centrifuged for 10 minutes at 3000 R P M Each tube is then cut with a file just above the kerosene xylene layer which is transferred to a special narrow cuvette for a first measurement of the absorptions at 460 mμ and 328 mμ These give values for carotene and for gross vitamin A

The solution is then transferred to soft glass tubes measuring 4 cm by 2 5–3 0 mm and is irradiated by a mercury arc lamp screened by a purple envelope, for 6–8 times as long as has been found necessary to destroy half the expected vitamin A (usually 30–60 minutes) The solution is then transferred back to the cuvette and a further reading is taken The net vitamin A is calculated from the difference between the two readings

By the use of special racks during saponification and irradiation it is possible for one analyst to make 50 estimations of carotenoids and vitamin A in a single day It is important to avoid the possibility of the development of slight turbidity after irradiation which will presumably increase the second reading and so diminish the apparent concentration of vitamin A For this purpose it is recommended that the pipette used to return the solution from the irradiation tube to the cuvette should be rinsed along part of its length, between each manipulation with anhydrous propionic acid

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CAROTENE IN RED PALM OIL

(CHAPTERS 6 and 30)

Production from the palm fruit

Both red palm oil and palm kernel oil which is valueless as a source of carotene are obtained from the fruit of the palm tree *Elaeis guineensis* of which a large number of sub species are known[1] Vast palm forests grow in West Africa but the palm is also cultivated in East Africa Malay Java and South America In good ground the trees begin to bear bunches of fruit when they are about five years old Each small fruit weighs about 6 5 g and consists of a stone or nut which is contained in a fleshy mesocarp or pulp The pulp weighs about 2 5 g the shell of the stone about 3 2 g and the kernel about 1 g The oils from the pulp and kernel are produced separately

According to traditional native processes the fruit are placed in a pit sprinkled with water and allowed to ferment The mass is then stirred round with sticks the oil skimmed off and the stones collected if the separation of their kernels is thought to justify the labour involved Oil obtained in this way is mainly exported for use in soap manufacture as also is the kernel oil For local use as food a special red palm oil known as chop oil is made by boiling the fruit with water The taste of the commercial red oil is generally considered unpleasant by Europeans but palm kernel oil is described as having a pleasant nutty flavour

Carotenoid contents

Extensive studies of the carotenoids in red palm oil were made by Hunter *et al*[2–6] in the laboratories of Lever Bros and Unilever Ltd Port Sunlight The main pigments found were invariably β and α carotenes but small amounts of γ carotene lycopene *neo*lycopene *neo*lutein and probably *neo* γ carotene were detected in some specimens The oil contents of the mesocarp and also the carotene contents of the oil appeared to increase with the maturity of the fruit The oil is remarkable for the high proportion of α carotene which is often found The results of analyses on typical oils obtained from fruits gathered at various stages of maturity[5] are given in Table 63

References p 590

TABLE 63

CAROTENE IN WEST AFRICAN RED PALM OIL
(ADAPTED FROM HUNTER AND SCOTT [5])

Variety	Maturity	% of oil in mesocarp	Carotenes $\alpha + \beta$ %	α Carotene % of total	Provitamin activity of oil i u /100 g
Afia oku	very immature	15 8	0 0473	43	62 000
	unripe	37 6	0 0597	30	85 000
	ripe	37 5	0 0634	41	84 000
	over ripe	55 8	0 0785	27	113 000
Lisombe	very immature	22 6	0 0165	42	21 000
	unripe	27 5	0 0496	43	65 000
	ripe	56 2	0 0726	40	98 000

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VITAMIN A IN FISH LIVER OILS AND OTHER MARINE OILS

(CHAPTERS 13 AND 30)

The wide variations between different fish in the vitamin A contents of the liver have been mentioned in the main text When it is recalled that during the second world war the price of soup fin shark livers touched 20 dollars per kg [1] the commercial importance of recognising those species having high reserves will be readily appreciated Doubtless the peak in the market has been passed now that synthetic vitamin A is readily available but some species are still important as sources of the vitamin

As a striking example of the enormous concentrations of vitamin A which can be accumulated in the livers we may turn to the data by Rapson *et al* [2] on the red steenbras (Table 71) Their richest specimen of the liver oil of this species contained about one third of its weight of vitamin A which implies that almost two thirds of the oil consisted of vitamin A esters In contrast certain sharks such as the basking shark and bonito shark (Table 68) and large rays (Tables 65 and 66) are poor sources of the vitamin It must be realised of course that the commercial value of the oil will depend not only on its potency but on the amounts which are available Thus cod liver oil is not a rich source of the vitamin A but is readily available in very large quantities

In addition to differences between species there has been ample evidence of variations mainly according to age and season in the same species Thus large old fish tend to have higher reserves of vitamin than small young fish In fish of the same age the amount of the liver oil may vary considerably with the season but the total amount of vitamin may remain almost constant Thus fish caught during late summer or winter may provide a high yield of oil of low vitamin content as opposed to a low yield but higher vitamin content during spring In Table 69 this general relationship appears to hold good for several of the species examined Pugsley Morrel and Kelly [3] found that in the Atlantic cod the vitamin A contents of the whole liver remains constant throughout the fishing season from June to October although the oil content of the liver increases The yield of oil per liver was greater in the resting than in the active stages of the spawning cycle In fish grouped as 4 6 years old the vitamin A contents of liver oil were greater than in fish 3 4 years old

In Tables 65–72 the order of listing the various fish usually follows that of the original authors but in Table 64 the fish have been rearranged according to the vitamin A potency of their liver oils

References p 598

TABLE 64

FISH LANDED IN BRITAIN OIL CONTENTS OF THE LIVERS AND SUGGESTED AVERAGE VITAMIN A CONTENTS OF THE OIL (LOVERN [4])

Species	*Oil % in liver*	*Av vitamin A i u /g oil*
Haddock	50–75	100
Whiting	50–75	100
Cod	50–75	1 000
Hake	50–75	1 000
Dogfish	50–75	1 500
Herring	10	5 000
Salmon	10	5 000
Halibut	15–20	50 000
Tunny	15–20	50 000

TABLE 65

FLORIDA SHALLOW WATER SHARKS AND RAYS CONTENTS OF LIVER OILS (SPRINGER AND FRENCH [5])

Common name	*Zoological name*	*Vitamin A i u /g liver oil*
Nurse shark	Ginglymostoma cirratum	641–6 720
Tiger and leopard sharks	Galeocerdo arcticus	1 375 4 760
Bull mullet and Salerno mackerel sharks	Carcharinus platyodon	1 812 43 375
Black nosed shark	Carcharinus acronotus	1 200
Sand bar shark	Carcharinus milberti	2 600 15 500
No name shark	Carcharinus falciformis	6 625
Silky shark	Carcharinus floridanus	1 712–5 375
Dusky shark	Carcharinus obscurus	6 500–58 500
Little black tip	Isogomphodon limbatus	4 250 22 250
Big black tip	Isogomphodon maculipinnis	2 092
Lemon shark	Hypoprion brevirostris	3 000 11 425
N Atlantic hammerhead	Sphyrna zygaena	34 250
Common southern hammerhead	Sphyrna biplana	5 400–137 000
Bonnethead	Sphyrna tiburo	900
Great hammerhead	Sphyrna tudes	8 250–340 000
Man eater or great white shark	Carcharodon carcharias	750–7 350
Sawfish	Pristis pectinatus	858–7 238
Spotted eagle ray	*Stoasodon narinari*	35
Cow nosed ray	Rhinoptera bonasus	675
Manta or devil fish	Manta birostris	284–418

TABLE 66

CUBAN SEA SHARKS AND RAY (ANGULO *et al* [6])

Common name	Zoological name	Vitamin A i u /g oil
Hammerhead shark	Sphyrna zygaena	3 300–60 600
Quimbombó		5 400–17 400
Jaquetón	Carcharias sp	3 250–13 400
Tiger shark	Galeocerdo arcticus	3 000–7 500
Spot fin ground shark	Carcharias limbatus	11 700
Cabezón		7 700
Smooth dogfish	Mustelus canis	5 300–5 700
Albacorera		2 800–4 800
Shark	Hexanchus sp	450–4 200
Nurse shark	Ginglymostoma cirratum	2 000–3 700
Sharp nosed mackerel shark	Isurus tigris	1 275–2 100
Man eating shark	Carcharodon carcharias	1 150
Elefante		850
Spotted whip ray	Batoidei sp	145

TABLE 67

INDIAN MARINE ESTUARINE AND FRESH WATER FISHES (SESHAN [9])

Type	Common name	Zoological name	Vitamin A i u /g oil
Marine	Saw fish	Pristis species	9 900
	Shark	Scolidon gangarius	1 600–4 600
Estuarine	Ilish	Hilsha ilisha	300–2 400
Fresh water	Arh	Mystus macronus	44 800
	Dhain	Silonia siluroid	38 400
	Boal	Wallago attu	19 200–20 800
	Shole	Ohiocephalus stratus	20 000
	Shillang	Silonia silompia	12 800–22 400
	Bhetki	Lates calcifer	7 300–8 600
	Mrigal	Cirrhina mrigala	5 100–8 800
	Pangash	Pangasius pangasius	7 200
	Kalbos	Labeo kalbasu	7 200
	Rohit	Labeo rohita	3 200–3 500
	Kätla	Catla catla	2 200–2 400
	Chital	Netopterus chitala	1 900

Most of the fresh water fishes showed an absorption maximum at 345 mμ (vitamin A_2) with an inflection at 290 mμ

References p 598

TABLE 68

FISHES OF THE OREGON COAST OIL AND VITAMIN A CONTENTS OF FISH LIVERS AND VISCERA
(SINNHUBER AND LAW [1])

Common name	*Zoological name*	*Part of body*	*% oil*	*Vitamin A i u /g oil*
Prickly bullhead	Cottus asper	Liver	9 6	3,150
Humpback salmon	Oncorhynchus gorbuscha	Liver	3	30,000
Gt blue shark	Prionace glauca	Liver	37	17,700
Mackerel shark	Lamna nasus	Liver	63 1	9,000
Three-toothed lamprey	Entosphenus tridentatus	Liver	20–30	12,000–20,000
Black cod	Anoplopoma fimbria	Liver	14–20	150,000–200,000
Pacific halibut	Hippoglossus stenolepsis	Liver	11–27	17,000–193,000
		Viscera	3 6	361,500
Soup-fin shark	Galeorhinus zyopterus, *m*	Liver	45–65	48 000–190,000
	f	Liver	51–70	10,000–68,000
Dogfish	Squalus suckleyi	Liver	61–81	5,000–34,000
Ling cod	Ophiodon elongatus	Liver	9–23	50,000–500,000
California pompano	Perilus simillimus	Liver	8–14	8 000–14,000
Ocean sunfish	Mola mola	Liver	49 8	454
Basking shark	Cetorhinus maximus	Liver	87	216
Lancetfish	Alepisaurus ferox	Liver	37	5,250
Columbia River chub	Mylocheilus caurinus	Liver	14 3	36,200
Columbia River smelt	Thaleichthys pacificus	Liver	12 2	32,600
		Viscera	5 3	81,000
Pacific hake	Merluccius productus	Liver	32 8	17,260
Pacific tomcod	Microgadus proximus	Liver	57 7	3,150
Blenny eel	Stickaedae sp	Liver	9 7	76,200
Midshipman	Porichthys notatus	Liver	15 9	6,830

Common name	Zoological name	Part of body	% oil	Vitamin A i u /g oil
Hagfish	*Polistotrema stoutii*	Liver	4–24	2 000–102 000
Chinook salmon	Oncorhynchus tshawytscha	Liver	8–10	25 000–40 000
		Viscera	*6 5*	21 000
Dover sole	Microstomus pacificus	Liver	7–19	5 000–31 000
		Viscera	0 5–1 5	1 000–3 000
Petrale sole	Eopsetta jordani	Liver	13–22	22 000–173 000
Black rockfish	Scorpaenidae sp	Liver	9–24	10 000 230 000
		Viscera	9 4	103 400
Red rockfish	Scorpaenidae sp	Liver	10–16	93 000–237 000
Olive backed rockfish	Sebastodes saxicola	Liver	5–10	36 000–115 000
		Viscera	2–7	*7 000–100 000*
Pacific mackerel	Decapturus sp	Liver	6 7	324 000
Shad	Alosa sapidissima	Liver	7 5	33 500
		Viscera	9	*15 000*
Turbot	Pleuronichthys decurrens	Liver	16–20	16 000–37 000
Thresher shark	Alopias vulpinus	Liver	37–39	1 000–17 500
Green sturgeon	Acipenser acutirostris	Liver	*24 0*	*1 610*
		Viscera	1 60	8 500
White sturgeon	Acipenser transmontanus	Liver	9–49	10 000–17 000
		Viscera	*2–5 5*	10 000–28 000
Albacore tuna	Germo alalunga	Viscera	2 9	3 260
Eel pout	Aprodon cortezianus	Liver	4 76	13 760
Bonito shark	Isurus glaucus	Liver	65	670
Skate	Rajidae sp	Liver	41 46	3 250
Ratfish	Hydrolagus colliei	Liver	83 87	600–900
Jap sole	Lyopsetta exilis	*Liver*	*5 6*	*71 800*

TABLE 69

BRITISH COLUMBIAN FLATFISH VITAMIN A IN RELATION TO THE WEIGHT AND OIL CONTENT OF THE LIVER AT VARIOUS SEASONS

(MCKERCHER [7])

Common name	*Zoological name*	*Liver wt range as % fish*	*Oil range % of liver*	*Vitamin A range i u /g oil*
Starry flounder	Platichthys stellatus	1 02 (Apr) 2 37 (Nov)	5 7 (Apr) 17 1 (Jul)	2,100 (Oct) 22 400 (Jul)
Dover sole	Microstomus pacificus	0 53 (Jan) 1 76 (Oct)	4 4 (May) 22 4 (Dec)	2,250 (Dec) 16 400 (May)
Sand sole	Psettichthys melanostictus	1 1 (Apr) 3 89 (Dec)	5 8 (Aug) 16 9 (Dec)	2,700 (Dec) 22 300 (May)
Rex sole	Glyptocephalus zachirus	0 65 (Apr) 1 48 (Oct)	5 8 (Apr) 23 8 (Dec)	1,400 (Oct) 11,650 (Apr)
Curl-fin sole	Pleuronichthys decurrens	1 55 (Jun) 2 36 (Apr)	3 8 (Jun) 10 9 (Apr)	6 250 (May) 13,500 (Jun)
Butter sole	Isopsetta isolepsis	1 20 (Sep) 1 39 (Jan)	8 2 (Jul) 15 7 (Sep)	7,600 (Sep) 11,200 (Jul)
C-O sole	Pleuronichthys coenosus	1 03 (Nov) 1 48 (Apr)	10 0 (Apr) 15 5 (Nov)	8,950 (Nov) 9,750 (Apr)
Brill	Eopsetta jordani	1 03 (Apr) 2 44 (Aug)	9 0 (May) 26 8 (Dec)	6,800 (Dec) 132 000 (Apr)
Rock sole	Lepidopsetta bilineata	0 93 (May) 1 88 (Dec)	2 5 (Jan) 13 7 (Aug)	2,900 (Dec) 20,400 (May)
Lemon sole	Parophrys vetulus	0 59 (Jan) 2 05 (Jul)	4 3 (Apr) 16 5 (Oct)	2,300 (Oct) 15,500 (Apr)

TABLE 70

WHALE-LIVER OILS BRITISH COLUMBIA

(SCHMIDT [8])

Species	*Av oil in liver, %*	*Av vitamin A i u /g oil **	*Standard deviation of potency*
Sperm	3 80	293,000	80,500
Humpback	3 84	130,000	14 400
Finback	3 50	45,900	16,800

* The estimations were made spectrophotometrically, with the application of the correction procedure of Morton and Stubbs on 3–6 specimens of each species The absorption maxima ranged from 322–325 mμ, which indicated that only a little kitol could have been present

TABLE 71

SOUTH AFRICAN FISH FROM CAPE AND NATAL WATERS SIZE OF LIVER PERCENTAGE OF OIL AND CONCENTRATION OF VITAMIN A IN OIL

(RAPSON *et al* [2])

Common name	*Species*	*Liver wt as % of fish*	*% of oil in liver*	*Vitamin A i u /g oil**
Yellow tail	Carangidae	1 0–1 2	0 7–11 4	61 400–236 000
Maasbanker	Carangidae	–	9 0–10 2	293 000
Elft	Pomatomidae	1 45–2 13	5 7–11 5	6 160–11 800
Harder	Mugilidae	2 8	11 6	3 200
Red steenbras	Sparidae	1–2 2	1 1–8 7	29 400–1 130 000
Mussel cracker	Sparidae	0 8	1 8–5 5	79 500–301 000
Seventy four	Sparidea	1 3 1 6	3 4–8 7	34 200 89 000
Scotchman	Sparidae	1 0	5 5	14 600
Silver fish	Sparidae	1 0	2 8	22 000
White steenbras	Sparidae	1 2	2 3	30 200
Red stumpnose	Sparidae	1 1	13 6	6 880
Variegated rock cod	Serranidae	1 3–2 0	4 0–4 4	37 000–110 000
Spotted rock cod	Serranidae	1 7–1 8	6 5–13 4	6 240 174 000
Galjeon	Not stated	1 1	5 5	162 000
Butter fish	Not stated	1 4	6–7 0	61 400–97 600
Monk fish	Not stated	2 2–4 7	26 0 36 5	4 640 15 800
King of herrings	Not stated	1 59	4 2	77 800
Milk fish	Not stated	8 5		21 000
Spotted grunter	Not stated	2 9	43 7	800

* Values for $E^{1\%}_{1cm}$ at 3 8 mμ given by the original authors have been multiplied by 1600

TABLE 72

AUSTRALIAN FISH VITAMIN A IN THE LIVER OILS

(CUNNINGHAM AND SLATER [10])

Common name	*Zoological name*		*Vitamin A i u /g %*
Snapper shark	Galeorhinus australis *f*		31 000
		embryos	130
Bonito	Sarda australis		670–1200
Small tunny	Euthynnus alletteratus		15 000
Albacore	Germo germo		1 700
Bluefin tunny	Thunnus maccoyii		8 400
Skate	Raja nasuta		600
Gummy shark	Mustelus antarcticus		670–2000

References p 598

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INDEXES

Author Index

Subject Index